RESPONSES OF PARABRACHIAL NUCLEUS NEURONS TO WHOLE-BODY MOTION IN THE MACAQUE

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ABSTRACT:

Projections from the vestibular nuclei to the parabrachial complex (PB) have been described in rats, rabbits, and monkeys, and have been proposed as a neuronal substrate for clinically-observed linkages between disorders of balance and of affect. This raised the questions of whether PB units respond to vestibular stimulation, and what details of whole-body motion are present in PB. The caudal two-thirds of the parabrachial and Kölliker-Fuse nuclei were explored by Balaban and coworkers (2002), and found to contain neurons responsive to whole-body, periodic rotations in vertical and horizontal planes. Responses to brief 'position trapezoid' stimuli indicated that PB units were sensitive to both angular velocity and static tilt, consistent with the presence of angular- and linear-acceleration sensitive inputs from the vestibular nuclei. In the majority of units, responses to brief static tilts (of 1.5s duration) appeared to reflect a sensitivity to linear acceleration in the head-horizontal plane, consistent with the presence of linear-acceleration sensitive inputs from the vestibular nuclei. We have replicated these results and further investigated the linear acceleration sensitivity of PB units using off-vertical axis rotations (OVAR). We have confirmed the general hypothesis that responses of many PB units to a rotating linear acceleration vector are consistent with the behavior of first- and second-order vestibular neurons. The majority of units responded to OVAR in a manner consistent with responses of vestibular neurons previously described as linear, one-dimensional accelerometers. Fewer units showed a variety of responses consistent with previously described central vestibular neurons suggestive of convergence of labyrinthine inputs with different spatial and temporal

response properties, as well as prominent ‘bias’ type responses consisting of significant changes in mean firing rate during rotation, in the absence of significant modulation.

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PREFACE

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1. Background and Introduction

The primary function of the peripheral vestibular sensory organs is to sense rotation and translation of the head in space. In the vestibular nuclei and other central vestibular sites, this information is integrated with visual and proprioceptive information from the retina and skeletal musculature, as well as oculomotor and skeletal motor commands, in order to allow the coordination of postural and oculomotor reflexes which facilitate the maintenance of postural stability and the stabilization of images on the retina. This integration also allows for the successful execution of intentional oculomotor and skeletal motor commands in both stable and unstable gravito-inertial or visual contexts, such as the interior of a rocking ship, or a darkened, crowded space lacking in major fixed visual reference points, by facilitating the discrimination of self-generated motion from motion that is exogenously imposed. The subjective sensation of self-motion and spatial orientation also derives from the vestibular end organs, in concert with visual and proprioceptive sensory systems.

When postural stability and spatial orientation are unchallenged, the functioning of the vestibular system is taken for granted. However, challenges to spatial orientation and failures of postural control (such as tripping and falling) in normal subjects result in both familiar acute affective responses (i.e. the acute anxiety and alerting responses to loss of balance) and the recruitment or diversion of attentional resources to the maintenance of balance and spatial orientation (Yardley et al., 2001; Gage et al. 2003), accompanied by changes in balance task performance (Nakahara et al. 2000; Adkin et al., 2002; Carpenter et al. 2004). Conversely, diversion of attention from vestibular or visual motion stimuli and changes in alertness are correlated with altered central vestibular function (Collins & Guedry, 1961; Collins, 1962; Magnusson, 1986).

In addition, individuals with compromised vestibular function frequently develop persistent affective symptoms related to visual and postural challenges to spatial orientation, and a spectrum of subtle vestibular dysfunctions have been identified in individuals with affective disorders (specifically, panic disorder with agoraphobia) (Guye, 1899; Jones, 1918; Jacob et al., 1985, 1993, 1996, 1997; Sklare et al., 1990, 2001; Yardley et al., 1994, 1995; Hoffman et al., 1994; Tecer et al. 2004; Jacob et al., 1995; Redfern et al., 2001). Vestibular and affective disorders are also associated with shared pharmaco-therapies (benzodiazepines and serotonin reuptake inhibitors) and positive responses to behavioral interventions (Jacob et al., 2001; Staab et al., 2002; Swartz & Longwell, 2005; Ramos, 2006). Finally, marked vestibular symptoms (dizziness, vertigo and incoordination—exacerbated by eye and head movements) are associated with "SSRI discontinuation syndrome," which occurs in some patients during withdrawal from these antidepressant drugs (Black et al., 1993; Coupland et al., 1996; Lejoyeux and Adès, 1997; Zajecka et al., 1997; Michelson et al., 2000; Bogetto et al., 2002).

Relationships between balance disorders and anxiety disorders have been recognized for hundreds of years. The historical literature regarding this association has been reviewed by Balaban and Jacob (Balaban & Jacob, 2001). Although these relationships are widely recognized, the neural mechanisms by which vestibular sensation, postural control and spatial orientation are linked to affective and cognitive brain functions have not been conclusively identified. However, a model was elaborated by Balaban and others (Balaban & Thayer, 2001; Balaban, 2002), which proposes that neurons of the PB (parabrachial complex, including the parabrachial and Kölliker-Fuse nuclei) are a key component of a network that connects the vestibular nuclei (VN) with brainstem and forebrain areas involved in autonomic, nociceptive and affective processing, particularly the extended amygdala and insular, infralimbic, and lateral

frontal cortex, as well as the thalamus and hypothalamus. This network was suggested as a neural substrate for clinically-observed linkages between balance disorders and disorders of affect, as well as normal affective responses to challenges to spatial orientation. However, prior to determining the specific functions (if any) of the parabrachial complex in this network, it is first necessary to establish the basic physiology of unit responses to vestibular stimulation.

1.1. Why do we expect to find vestibular responses in the parabrachial complex?

Two major findings suggest that units in the parabrachial complex are sensitive to sensory inputs from the vestibular labyrinth: 1) anatomical connections exist between the vestibular nuclei and the parabrachial complex, and 2) parabrachial units show responses to passive whole-body motion. These findings are briefly summarized here, and examined further in the following sections.

First, anatomical studies in rats and rabbits (Balaban 1996, 2004; Porter and Balaban 1997), as well as limited confirmatory data in monkeys (Balaban et al., 2002), have found vestibular nuclear (VN) efferent fibers terminating in a broad region of the parabrachial and Kolliker-Fuse nuclei. In addition, reciprocal projections to VN from PB were found to innervate a larger region of VN than the region of VN observed to project to PB. Second, responses to whole-body rotations have been observed in PB units (Balaban et al., 2002). These responses appeared to reflect unit sensitivities to angular velocity, consistent with inputs from the vertical and horizontal semicircular canals, and to linear accelerations in the head-horizontal plane, produced by head tilts with respect to gravity, consistent with inputs from the utricle.

Prior to reviewing these findings, we will briefly review the anatomical organization of PB and the organization of vestibular nuclear efferent innervation of PB, and summarize the responses of these central vestibular areas to self-motion.

1.1.1. Location and subdivisions of the parabrachial nucleus

The Kolliker-Fuse nucleus is a magnocellular region located at the ventrolateral edge of the margin of the brachium conjunctivum. The parabrachial nucleus, or marginal nucleus of the brachium conjunctivum, can be described as a parvocellular region of cells contiguous with the Kolliker-Fuse nucleus, and extending dorsomedially to surround the brachium. Thus, the PBN-KF complex appears as a region of ventrolaterally dense and dorsomedially sparse neurons surrounding the dorsal, ventral, and lateral margins of the brachium conjunctivum. The regions of PBN located dorsolateral and ventromedial to the fibers of the were so named in the cat (Taber, 1961) in recognition of the more vertical orientation of the PBN in humans (Berman 1968; Lavezzi et al., 2004).

PBN has been further parcellated into several subnuclei with distinct cytoarchitechtonics nd/or connectivity, with significant differences recognized between species. Fulwiler and Saper (1984) are the most frequently cited regarding parabrachial subdivisions. They distinguished, in the rat, seven subdivisions of the lPBN, and two divisions of the mPBN, in addition to the KF nucleus. These divisions are summarized in **Table 1.1** and **Figure 1.2**. The efferent connections of these divisions are discussed in Chapter 3.

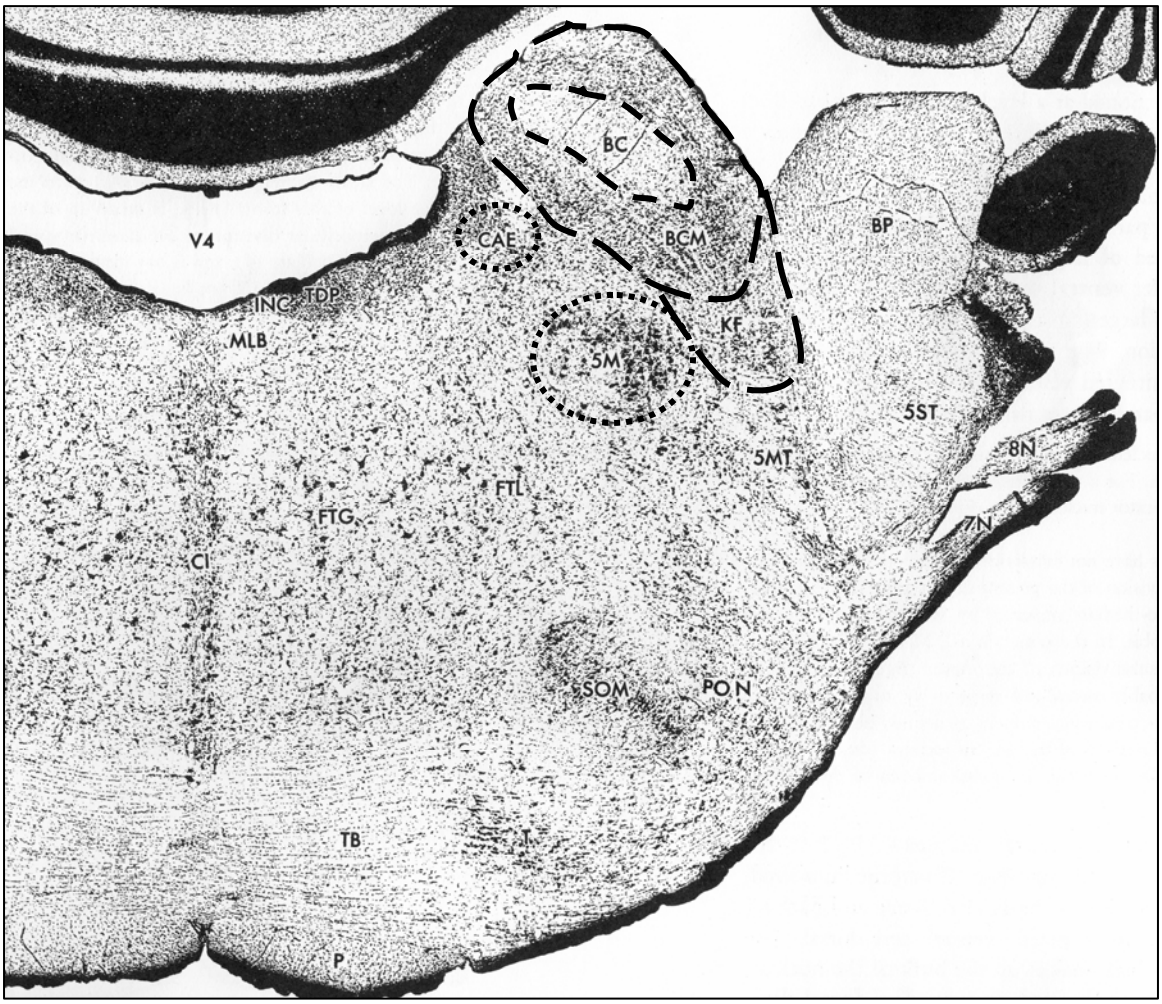

Figure 1.1 The Parabrachial Nucleus: Frontal Section

The location and basic organization of the PB is well presented in this frontal section from the cat (adapted from Berman, 1968). This figure was chosen because it clearly shows the relationships between the ventrolaterally-situated Kolliker-Fuse (KF) nucleus, the lateral (dorsal) and medial (ventral) PB nuclei (labeled BCM) as they surround the brachium conjunctivum (BC), as well as the mesencephalic trigeminal nucleus (5M) ventrally, and locus coeruleus (CAE) medially. Note the continuity of PBN and KF ventrolaterally, the relative thickness of PBN dorsal and ventral to BC, and the gradient from dense to sparse packing of units as one moves dorsomedially in the PBN-KF complex.

In contrast to the species-specific study of subdivisions of the rat PB by Fulwiler and Saper, Petrovicky and Kolesarova (Kolesarova & Petrovicky, 1987; Petrovicky & Kolesarova 1989; Petrovicky, 1989) examined the comparative anatomy of PB in several bird and mammal species, and in man. They proposed a simplified scheme of PB subdivisions compared to that of Fulwiler and Saper, and found significant differences between PB in rodents, primates and humans. The PBN of humans is greatly enlarged compared with other species, and is rotated

vertically, filling the lateral third of the pons at the ponto-mesencephalic junction. Subnuclei DL, L, and M are largest, relative to other subnuclei, in man. Subnucleus D is absent in primates and man, while Ve is said to be absent in man and is proposed to be the most primitive portion of PBN, becoming progressively smaller in more advanced vertebrates.

Fulwiler & Saper (1984)		**Petrovicky & Kolesarova (1987)**	
m	medial	M Ve	Medialis Ventralis
exm	external medial	K-F	Kolliker-Fuse, pars caudalis
c	central lateral	DM	dorsomedialis and its cell condensations
v d s	ventral lateral dorsal lateral superior lateral	DM	
i	internal lateral	D	dorsalis
e	external lateral	L DL	lateralis dorsolateralis
kf	Kollker-Fuse	K-F	Kolliker-Fuse

Table 1.1 Comparison of naming schemes for PB subdivisions in the rat

Kolesarova and Petrovicky's description of PB subdivisions provides a simplified naming scheme, which serves as the basis for their later comparative study. It is important to note that these subdivision schemes were based primarily on cytoarchitectonics, and bear only approximate relationships to parabrachial afferent and efferent connectivity.

Kolesarova and Petrovicky also recognized that the nucleus originally described by Kolliker and Fuse is today referred to as pars compacta of the pedunculopontine nucleus, which is more cell-dense and located differently from the K-F nucleus of recent authors, and that the "K-F nucleus" nucleus described by recent authors as being quite prominent in the rat, cat, and monkey (see above for references) is *not* found in the adult human (Petrovicky, 1989; Kolesarova & Petrovicky, 1987). The confusion results from similarities in cell density and the size of these nuclei when comparing tissue from man to cats or rodents, and the absolute (but not relative) position of Kolliker and Fuse's nucleus in the human compared to the location of recent authors' "K-F nucleus" in other mammals. Kolliker and Fuse's nucleus is located lateral to the central part of the vertically-oriented PBN in man, while the "K-F nucleus" of recent authors is located

at the ventrolateral margin of PBN. When referring to "K-F" in this document, we are referring to the nucleus described by recent authors.

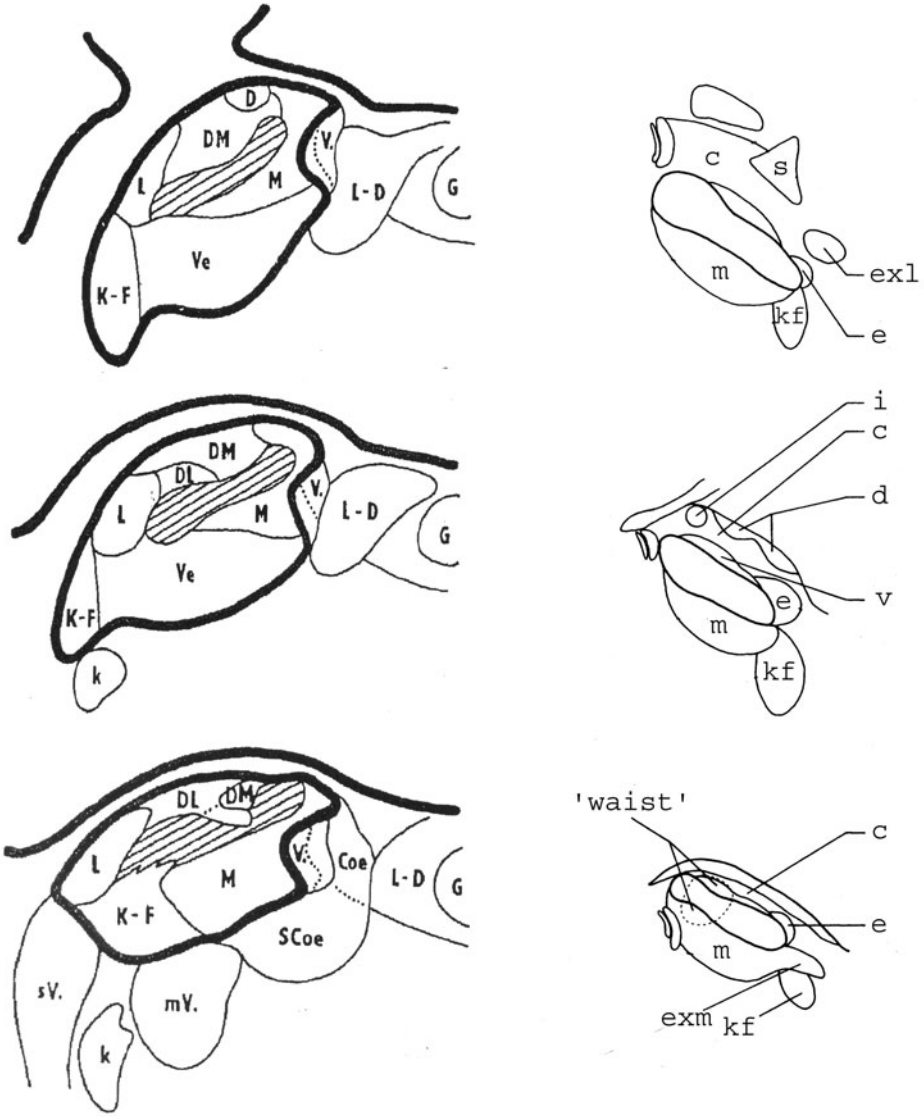

Figure 1.2 Comparison of PB subdivision schemes

Left panels: Kolesarova and Petrovicky's normalized scheme for cross-species subdivisions of PB. *Right panels*: Fulwiler and Saper. *Upper panels:* rostral. *Lower panels:* caudal. See text for discussion.

Kolesarova and Petrovicky's studies have been virtually ignored in the animal literature (see, e.g. Kitamura et al., 2001), but raise concerns about the extreme subdivision scheme proposed by

Fulwiler and Saper when applied to primates. Rather than being confined to particular subnuclei as defined by cytoarchitectonic features, responses of single units in PB and the organization of afferent and efferent pathways in PB tend to be distributed in a gradient or punctate fashion across two or more of Fulwiler and Saper's subdivisions, including subunits located on opposite sides of the brachium as well as sparse cells interdigitated within the brachium itself. In fact, the minutia of PB subdivisions may in some cases be better accounted for by the course and subdivisions of the brachium itself than by minor cytoarchitechtonic differences between spatially segregated cell groups within the nucleus (Kitamura et al., 2001). Accordingly, it is not surprising that motion-sensitive neurons were distributed throughout a large portion of PB (Balaban et al., 2002), rather than being concentrated in particular subnuclei. Nevertheless, we have used Fulwiler and Saper's terminology (as applied to the monkey by Kitamura et al., 2001) as this is germane to the cited literature.

1.1.2. Vestibular-parabrachial interconnections

Vestibular nuclear projections to PB were first noted by Balaban (Balaban, 1996), using anterograde and retrograde tract-tracing methods. In the rat and rabbit, the vestibular nuclei give rise to significant, bilateral projections to approximately the caudal two-thirds of the lateral, dorsal, and ventral portions of PB, including the Kolliker-Fuse nucleus (Balaban, 1996, 2000; Porter and Balaban, 1997). In particular, the superior and inferior vestibular nuclei, and the caudal part and intermediate third of the medial nucleus give rise to bilateral projections to the medial, external medial, external lateral, and ventral lateral parabrachial subnuclei, and to KF.

Recent evidence (Balaban, 2000, 2004) showed that the caudal regions of PB which receive vestibular nuclear efferents send bilateral projections to VN. In return, caudal portions of the ventral and lateral parts of PB send projections primarily to the ipsilateral superior, medial,

and lateral vestibular nuclei, with fewer bilateral projections to all of these regions (Balaban, 2004). In particular, PB efferents innervate the superior and inferior vestibular nuclei, and the rostro-dorsal and caudal parts of the medial nucleus, and the caudo-ventral and intermediate parts of the lateral vestibular nuclei. Double-retrograde tracing experiments have also observed collateralization of PB projections to VN and CeA (AL Halberstadt & CH McCandless, unpublished observations). In addition, PB receives projections from ipsilateral cerebellar vermis lobule IX, which also gives rise to the largest population of cerebellar corticovestibular projections (Haines, 1975), suggesting the potential for convergence of self-motion-related inputs to PB from multiple central vestibular sites.

Projections from VN to the PBN-KF complex appear to be arranged in a weakly gradient fashion, with more caudoventrolateral regions receiving higher-density inputs, and more rostrodorsomedial regions receiving fewer inputs, although the portion of mPBN abutting the brachium appears to receive slightly more dense inputs than neighboring regions, similar to the density of VN projections to KF. Because vestibular nuclear axons terminating within PBN frequently appear to branch and to give rise to *boutons-en-passage* (Balaban, 1996; Balaban et al., 2002), the functional efficacy of these projections may be greater than the sparse appearance of anterograde labeling in PB might suggest.

1.1.3. Responses of units in vestibular nuclear regions innervating PB

The peripheral vestibular organs in each labyrinth are comprised of three angular acceleration-sensitive organs (the anterior, posterior, and horizontal semicircular canals), and two linear acceleration sensors (the utricle and saccule). The anterior and posterior semicircular canals are vertically oriented and arranged at approximately 90° angles on each side of the head, providing peak sensitivity to angular accelerations in vertical planes. Each organ on one side of the head is

paired with an organ oriented in the same plane on the opposite side of the head. The LARP (left-anterior/ right-posterior) canal plane crosses the head between the nose and left ear on the left side, and between the right ear and the occiput on the right side of the head. The RALP (right-anterior/ left-posterior) plane crosses the head between the nose and right ear on the right side, and between the left ear and occiput on the left side. Rotation in the LARP plane maximally stimulates the left-anterior and the right-posterior vertical semicircular canals, and rotation in the RALP plane stimulates the right-anterior and left posterior vertical canals. The horizontal semicircular canals are slightly inclined rostrally, such that maximal stimulation of the horizontal canals is achieved during yaw-axis rotation with the head tilted slightly nose-down (approximately 20-25°) from the stereotaxic horizontal plane. The firing rates of primary afferents in the VIIIth nerve innervating each semicircular canal are increased by rotation toward the side of the canal, and decreased by rotations away from the canal, such that e.g. yaw-axis rotations toward the left ear increase the firing rate of left horizontal semicircular canal afferents and decrease firing rates in right horizontal canal afferents. Responses of semicircular canal afferents to transient motions encode an estimate of head angular velocity, however, the effective stimulus to which the canals and primary afferents are responsive is angular acceleration, rather than angular velocity (Goldberg & Fernandez, 1971), due to mechanical transduction of angular acceleration to angular velocity by the semicircular canals (van Egmond et al., 1949). Several response types are recognized among rotation-sensitive units in the vestibular nuclei (Fuchs & Kimm, 1975; Chubb et al., 1984; Scudder & Fuchs, 1992). The majority of canal-related vestibular nucleus units are also responsive to movement of the visual surround (Waespe & Henn, 1977) as well as showing prominent relationships with eye movements. Other units are responsive only to rotations, and are unrelated to eye-movements. Semicircular canal afferents

terminate primarily within the superior and medial vestibular nuclei, with fewer inputs to the lateral and inferior nuclei (Gacek, 1969).

Whereas hair cells in the cupulae of the semicircular canals are sensitive to angular accelerations, hair cells in each otolith organ are arranged to respond to all directions of linear acceleration in the plane of the organ. The utricle is oriented approximately horizontally and senses acceleration in the head-referenced horizontal plane. The saccule is oriented approximately vertically, and senses linear acceleration in the saggital plane. Each macula is divided by a curved central line called the striola. Individual otolith primary afferents in the VIIIth nerve innervate small portions of the otolith maculae. Hair cells that respond best to oppositely directed linear accelerations are arranged opposite to one another on either side of the striola, so that linear accelerations in any direction in the plane of the macula both excites and inhibits some proportion of macular hair cells (Goldberg & Fernandez, 1975; Goldberg et al., 1990).

Orientation vectors of vestibular afferents which innervate the utricular macula are organized in an approximately earth-horizontal plane when the head is held upright as when walking with gaze directed several paces ahead, or in a plane which is tilted upward by approximately 20-30º rostrally compared to the stereotaxic horizontal plane (Loe et al., 1973). Responses in the inferior branch, which innervates the saccular macula, are responsive to translations in the saggital plane, with equal numbers of responses to both upward and downward vertical translations, and fewer responses to anteroposterior accelerations, consistent with the more linear, primarily head-horizontal orientation of the saccular striola in an approximately parasaggital plane (Fernandez & Goldberg, 1976).

Utricular afferents primarily innervate the caudal, ventral, and lateral portions of the vestibular nuclei (Siegborn & Grant, 1983; Peterson, 1970), with the majority of inputs targeting the descending vestibular nucleus, the ventral half of the lateral nucleus (Deiter's nucleus), and the caudal aspect of the medial nucleus (Peterson, 1970). It is estimated that 66-80% of primary utricular afferents and second-order units in the vestibular nuclei respond best to ipsilateral-ear down tilt (Adrian, 1943; Fujita et al., 1968; Curthoys & Markham, 1971; Peterson, 1967, 1970; Loe et al., 1973; Fernandez & Goldberg, 1976), with the majority of remaining units excited by contralateral-ear down tilts. Responses of utricle-sensitive vestibular nucleus units to nose-down and nose-up (occiput-down) tilts are less pronounced (Peterson, 1967, 1970; Curthoys & Markham, 1971). This distribution of response vectors in central vestibular units results from the distribution of hair cell polarization vectors in the utricle (Goldberg et al., 1990) and the ipsilateral projection patterns of utricular primary afferents, as midline sections disrupting commisural projections do not affect the proportion of units of each type encountered (Curthoys & Markham, 1971). However, a push-pull arrangement of commissural inputs from opposite utricular maculae seems to reinforce this pattern, as experiments in unilaterally-labyrinthectomized animals (Fujita et al., 1968) show the same proportion of response types in the vestibular nuclei ipsilateral to the lesion.

Additional response types in utricle-sensitive vestibular nuclear units have been described as positively rectified (excitatory for bidirectional tilts) or negatively rectified (inhibitory for bidirectional tilts) (Peterson, 1967, 1970; Fujita et al., 1968). Such responses become more common in second and third-order neurons such as those found in the oculomotor nucleus (Azzena, 1966), and have been suggested to result from the convergence of first or second-order

vestibular nucleus units with unrectified responses to tilt onto second or third-order units (Peterson, 1970; Curthoys & Markham, 1971; Goldberg & Fernandez, 1971).

1.2. What responses do we expect to find in PB?

1.2.1. Previously described responses to position trapezoids:

Balaban and coworkers (Balaban et al., 2002) recorded from PB neurons during periodic whole-body motions, using a 'position trapezoid' stimulus consisting of brief rotation transients interleaved with brief periods of static position, or static tilt. This stimulus and the model proposed by Balaban et al. (2002) to account for responses to it (see **Methods**, **Chapter 2**) are complex, and are treated here only in the abstract.

Importantly, although the proposed model is able to fit the observed unit responses as being due to sensitivity to angular velocity and static tilt, it is not possible to attribute each response component unambiguously to specific labyrinthine inputs. Therefore, it is necessary to study the angular velocity and linear acceleration responses of these units separately. The present study first replicates the major findings of the previous study with respect to position trapezoid stimulation, then proceeds to the investigation of linear acceleration-like responses by testing unit responses to a rotating linear acceleration stimulus vector produced by rotation about an axis which is tilted with respect to the gravity vector.

Position trapezoid stimuli were used here in order to first replicate the findings of Balaban et al. (2002), prior to further investigation of a single component of unit responses to this stimulus. Theoretically, the position trapezoid stimulus has the advantage of containing discrete angular velocity and static position segments (**Figure 1.3**).

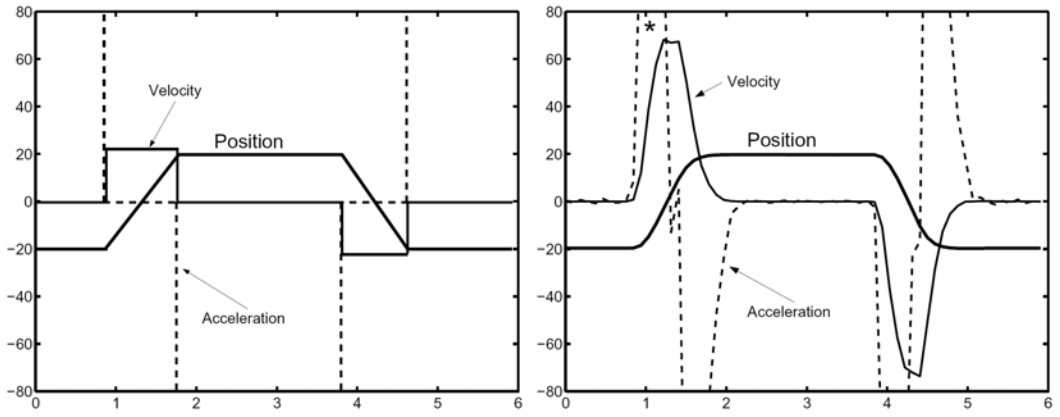

Figure 1.3 Ideal and actual position trapezoids

The previous study (Balaban et al., 2002) used position trapezoid stimuli to elicit both angular velocity-like and static tilt-like responses from PB units. An ideal position trapezoid stimulus profile (*left panel*) provides discrete acceleration, velocity, and static position segments, to which unit responses may be correlated. In practice, it is extremely difficult to produce brief position trapezoids which succeed in separating angular acceleration, velocity, and position into discrete time periods. Rather, angular velocity and angular accelerations are confounded (*right panel*), making it difficult to interpret unit responses to this stimulus. It is reasonable, however, to hypothesize that responses to rotation transients reflect a sensitivity to angular velocity, and that responses to brief static tilts result from sensitivity to head-horizontal linear accelerations.
*Peak acceleration was approximately 270º/s^2.

Assuming an ideal motion profile, position trapezoid stimuli might allow the identification of unit response components to static tilt and angular velocity for units with convergent canal and otolith inputs. For example, trapezoidal stimulus profiles in which the trapezoidally-varying parameter is velocity have been used successfully to disambiguate responses to angular velocity and acceleration. Goldberg & Fernandez (1971) used long-duration velocity trapezoids to demonstrate that the effective stimulus to which semicircular canal afferents are responsive is angular acceleration, rather than angular velocity. They rotated animals in semicircular canal planes, using a stimulus consisting of a period of constant angular acceleration, followed by a period of constant angular velocity. A second period of constant deceleration returned the animal to zero angular velocity. Importantly, each period was of sufficient duration to allow unit responses to stabilize. Responses of canal afferents to this stimulus consisted of prominent responses to acceleration and deceleration, followed either by a return to baseline firing, a brief

under- or overshoot of baseline firing, or a slow decay to baseline firing rate, depending on the magnitude of the imposed acceleration.

Later, Fernandez & Goldberg (1976a,b) also studied the responses of otolith afferents to linear accelerations by using both static tilts and centrifugal force trapezoids. The centrifugal force stimulus was produced by mounting the animal one foot off of the axis of table rotation. By applying a velocity trapezoid input to the driven axis, the centrifugal linear acceleration force produced at the end of the rotating arm first increased at a constant rate, then was held constant for an arbitrary period, and finally returned to zero with a constant rate of deceleration. These studies showed that afferents in the superior division of the vestibular nerve, innervating the utricle, and afferents in the inferior division, innervating the saccule, responded to constant linear accelerations with sustained changes in firing rate, but often showed larger, transient responses during the transition periods when the applied force was changing.

Studies using velocity and acceleration trapezoids benefit from the fact that it is feasible to produce long-duration constant angular accelerations with available motors and motion-control circuitry. However, production of true *position* trapezoids, with distinct constant-velocity and static-position segments, would require the ability to produce infinite angular accelerations, or at least very brief, high-acceleration pulses, at the transition between static position and constant-velocity rotation. This requirement was not met in the previous study (Balaban et al., 2002), nor in the current study. However, the aim of the current study is not to definitively characterize the angular velocity-related responses of PB units, but to investigate the suggestion from position trapezoid responses that many PB units are sensitive to static tilt or linear acceleration in the head-horizontal plane. Therefore we have focused primarily on the orientation

component of angular velocity-like responses to position trapezoids, and have used OVAR to further investigate the static tilt components of unit responses to position trapezoids.

The components of the response model introduced by Balaban et al. (2002) are shown in **Figure 1.4**. This model includes response components due to angular velocity, a leaky integration of angular velocity, and static position. The detailed model equation is given in **Chapter 2**; the discussion here is necessarily more abstract.

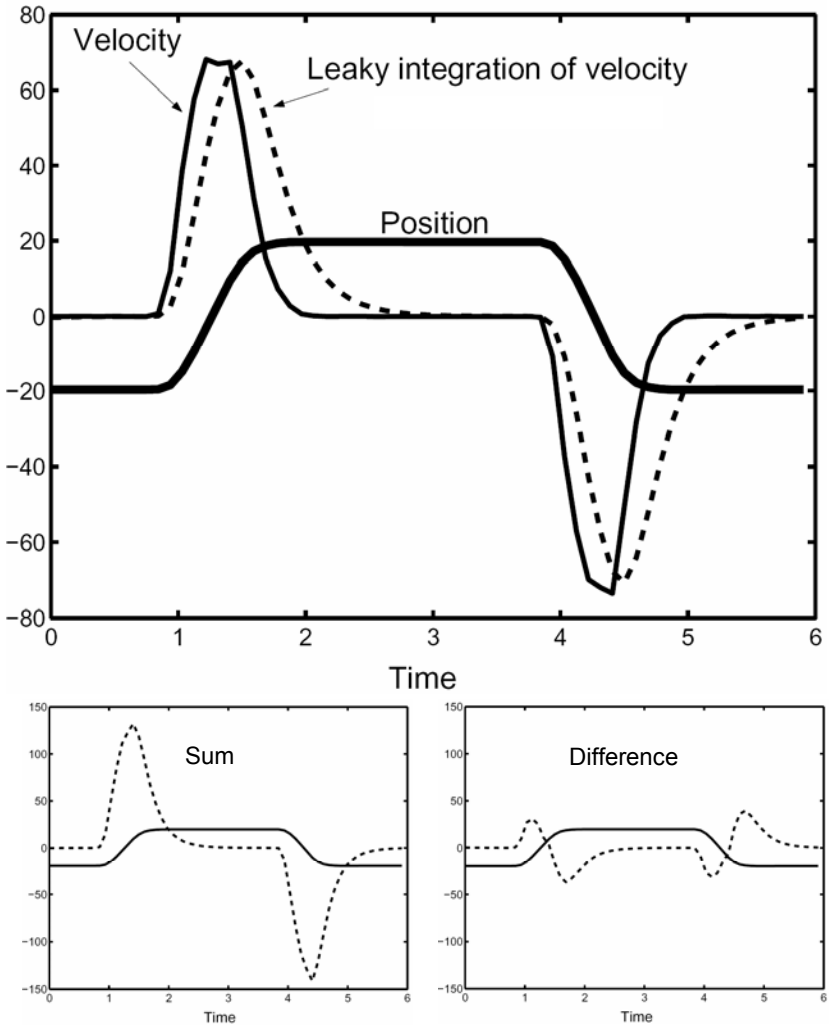

Figure 1.4 Position trapezoid response model components

Upper panel shows the response components included in the model of position trapezoid responses used by Balaban et al. (2002). Heavy trace: whole-body position. Light trace: whole-body angular velocity.

Dashed trace: Leaky integration of angular velocity. Lower panels: hypothetical responses of a unit with velocity and integrated velocity response components with the same polarity (left; sum of velocity and integrated velocity traces in upper panel) and with opposite polarity (right; difference of velocity and integrated velocity).

The response component modeled previously as a leaky integration of angular velocity (Balaban et al., 2002) may in fact be due either to angular acceleration sensitivity or sensitivity to dynamic changes in the applied linear acceleration force due to gravity during tilt transitions (discussed further in **Chapter 2**). Further study is necessary to disambiguate this response component. Importantly, the angular velocity and leaky integrated velocity components of this model are not temporally distinct. Therefore, model fits to unit responses which include significant fractions of both of these components may greatly over- or underestimate the contribution of either component to the total response (**Figure 1.5b**). For this reason, Balaban et al. (2002) chose arbitrary, fixed time-constants for the 'leaky integrator' response model, which allowed the description of the greatest number of unit responses. While time-constants might have been estimated for each unit response, inspection of model fits (**Figures 1.5** and **2.5**) shows that this approach might result in significant confusion regarding estimates of responses to angular velocity. For example, the response shown in **Figure 1.5b** was fit by a model with nearly equal velocity and integrated velocity gain coefficients (dashed lines). Fits to the response using only one or the other component are also shown (solid lines), demonstrating the ambiguity inherent in the model. Other responses which are successfully fit by this model are equally difficult to interpret. For example, it is difficult to state definitively whether a biphasic response to angular rotation transients (**Figure 1.4**, lower right; **Figure 1.5d**) is due to a combined sensitivity to both angular velocity and leaky integrated angular velocity with opposite signs, or to a sensitivity to angular acceleration.

In the absence of further data which might allow the disambiguation of these transient response components (such as the velocity trapezoids used by Goldberg & Fernandez (1971) to disambiguate angular acceleration and velocity responses of canal afferents), it is only possible to specify a single orientation vector describing the plane of rotation producing the greatest velocity-like response to rotation transients. While the responses of parabrachial units to angular rotation transients *suggest* that these units are sensitive to angular velocity or acceleration, separate studies will be necessary to confirm these results.

1.2.2. Goals of the study:

The study published by Balaban et al. (2002) provided the first evidence that units in the parabrachial complex respond to whole-body motions using a compound stimulus which included both angular velocity and static tilt components. The present study represents the first step in disentangling the complex responses to position-trapezoid stimulation described above. These experiments used off-vertical-axis rotations to produce a rotating linear acceleration stimulus in the head-horizontal plane. This type of stimulus has been used previously to characterize the responses of central vestibular neurons to linear accelerations. Study of responses to this stimulus will improve our understanding of linear acceleration sensitivity in neurons of the parabrachial complex—a property which was first suggested by responses to the more complex vertical-plane position trapezoid stimulation. Responses to OVAR which are similar in character and distribution to OVAR responses that have been reported in other central vestibular units will support the hypothesis that self-motion responses in neurons of the parabrachial complex respond to linear acceleration inputs derived from the vestibular labyrinth.

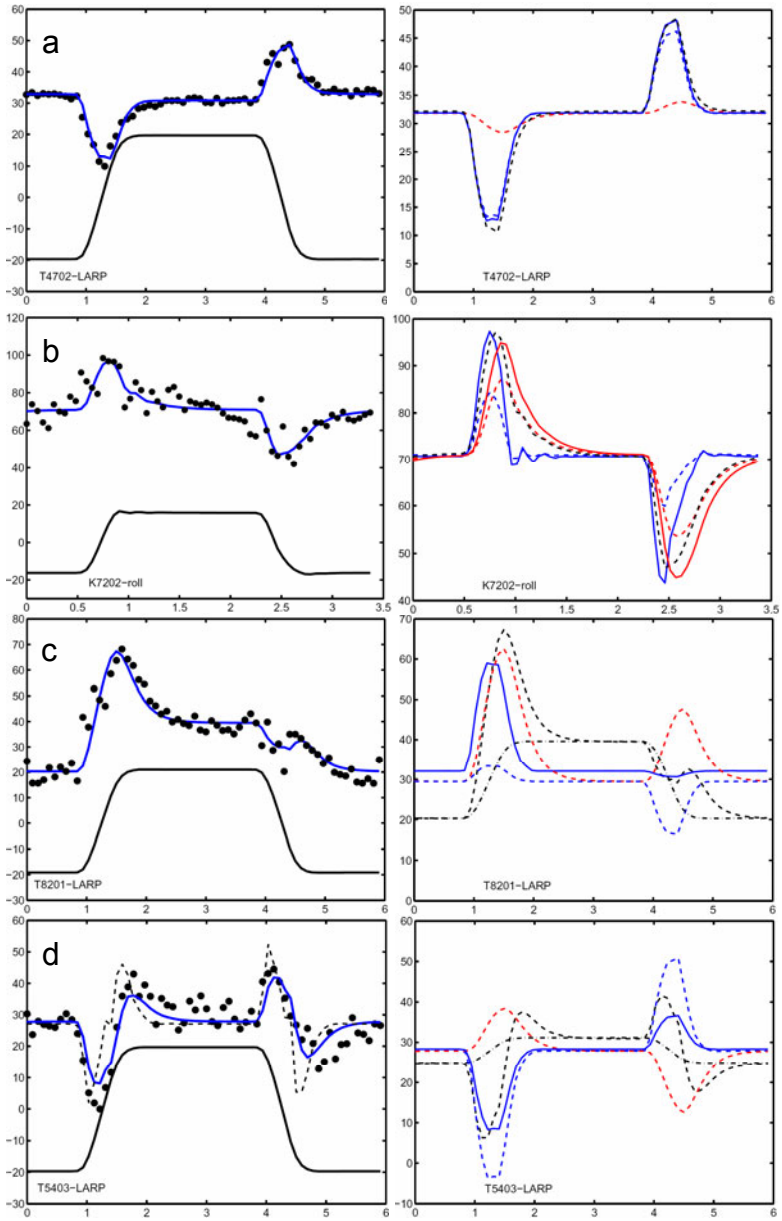

Figure 1.5 Example fits according to the model proposed by Balaban et al.

Responses of four example units to position trapezoid stimulation in vertical planes. *Left column*: unit responses and model fits. *Right column*: individual components of each model fit. See text for explanation. ***a***: Unit responding to angular velocity. ***b***: unit responding to angular velocity and an apparent leaky integration of angular velocity. ***c***: unit responding to integrated angular velocity and static tilt. ***d***: unit responding to angular velocity, integrated angular velocity, and static tilt. *Right column*: *dashed black line*: complete model fit as given in left panels; *dashed blue line*: angular velocity component of complete model; *dashed red line*: integrated angular velocity component of complete model; *solid blue line*: best velocity-only fit to the data; *solid red line*: best integrated velocity-only fit to the data; *secondary dashed black trace* in right panels of ***c*** and ***e***: static position component of complete model. *Dashed trace in left panel of* ***d***: best fit acceleration-only model of unit response.

2. EXPERIMENTS

2.1. SUMMARY AND CONCLUSIONS:

1. A population of neurons responsive to whole-body motion was recently discovered in the caudal parabrachial complex (PBN) of alert monkeys by Balaban and coworkers (Balaban et al., 2002). This finding was consistent with anatomical studies which found vestibular nuclear efferent fibers terminating in these regions (Balaban 1996, 2004; Porter and Balaban 1997). Prior work examined the responses of PB neurons to whole-body rotations in three-dimensions and to brief static tilts, using position trapezoid stimuli. Results from brief static tilts suggested that a majority of recorded units were sensitive to linear accelerations in the head-horizontal plane (Balaban et al., 2002). The present study examines linear acceleration sensitivity in 103 neurons recorded from the left PB through the analysis of responses to a rotating linear acceleration vector produced by off vertical axis rotations (OVAR) over a narrow frequency range, and compares these to results from position trapezoid stimulation.

2. The major characteristics of unit responses to trapezoidal motion profiles described by Balaban et al. (2002) were replicated. These results were then compared with responses to OVAR in the same units. The linear-acceleration responses of PB units as assessed with OVAR stimuli differed from responses to brief static tilts in the same units in terms of both response gain estimates and preferred stimulus orientations.

3. The majority of unit responses (n=65) to OVAR demonstrated a perstimulus modulation in firing rate. Most of these units (n=48) could be characterized as one-dimensional, linear accelerometers, consistent with responses of primary vestibular afferents and many central vestibular responses. Additional response types were consistent with responses of central vestibular neurons receiving convergent input from two or more vestibular units with different

spatial and temporal response characteristics. A third response type consisted of 'bias' responses consisting of increases or decreases in mean firing rate related to the direction of rotation, in the absence of modulation responses.

4. The distribution of orientation vectors of one-dimensional PB units was consistent with that seen in primary afferents and central vestibular units. The largest proportion of cells (21/48) had preferred stimulus orientations within 45º of ipsilateral (left) ear-down tilt, with fewer cells responding to contralateral ear-down tilts and tilts in the pitch plane.

5. Most one-dimensional units (n=34) showed moderate phase leads (near 45º on average) during rotation. Response orientations, gains and phases tended to be stable over the limited frequency range tested.

6. In contrast to vestibular nuclear units, orientation vectors of PB units showed a differential anatomical distribution. Units in the left PB with orientations near left ear-down tilt were located ventral to units with orientations near right ear-down tilt.

7. The PB is a complex structure which participates in multiple ascending and descending pathways, including those involved in nociception, general visceral sensation, gustation, learning and visceromotor and autonomic control. Findings of anatomical linkages with the vestibular nuclei have been interpreted as evidence for the involvement of PB in observed interactions between balance and affect. It is concluded that responses of PB units to a rotating linear acceleration vector produced by OVAR are consistent with the existence of functional input from otolith-sensitive neurons in the vestibular nuclei.

2.2. Background

Recent electrophysiological evidence indicates that many neurons in the caudal PB respond to whole-body rotation and static tilts (Balaban et al., 2002). These findings were in agreement with anatomical evidence of projections from the medial, inferior, and superior vestibular nuclei to the PB (Balaban, 1996, 2000; Porter and Balaban, 1997; Balaban et al., 2002). In addition, immediate-early gene expression is induced in caudal PB cells by exposure to altered gravitational environments, although this effect may be due to non-vestibular inputs (Dit Duflo et al. 2000, Murakami et al. 2002, Fuller et al., 2004).

Balaban and coworkers (Balaban et al. 2002) recorded from single units in caudal two-thirds of the primate PB, while stimulating with whole-body rotations in several vertical and horizontal planes. That study used a 'position trapezoid' stimulus profile, consisting of brief periods of angular rotation at constant-velocity (~0.25s) interleaved with a brief period (~1.5s) of static yaw angular position or static tilt. Unit responses were accounted for with a model which included response components related to angular velocity, horizontal plane linear acceleration (i.e. static tilt sensitivity), and what they referred to as a "leaky integration of angular velocity" (conceptually, this is nearly equivalent to a high-pass filtered representation of angular position with some additional gain parameter to match the magnitude of angular displacement (position) to the magnitude of the angular velocity producing that displacement) in vertical rotation planes, and/or to angular velocity and leaky-integrated angular velocity during yaw-axis rotations. Each of these response components were spatially tuned, showing a preference for rotations in particular planes of tilt and/or sensitivity to yaw-axis rotation. The presence of angular velocity sensitivity suggested that PB units receive input from semicircular canal-sensitive neurons in the vestibular nuclei. The study also found that in 60% of units, subtraction of the response components modeled as being due to angular velocity and a leaky-integration of angular velocity

failed to account for the entire response, leaving a residual consisting of a DC offset in firing rate corresponding to the duration and magnitude of vertical-plane angular displacement, i.e. a static-tilt response, with some preferred orientation (Balaban et al., 2002). This finding indicated a probable contribution of otolith-derived vestibular inputs to the observed responses.

The study presented here extends the analysis of PB responses to linear acceleration using OVAR. Constant velocity OVAR produces a rotating linear acceleration stimulus proportional to the angle of tilt relative to gravity, in the absence of angular acceleration. This stimulus is discussed in detail in **Section 2.4.1**, below.

2.3. Hypotheses:

We expected the OVAR responses of most units to conform to the one-dimensional linear accelerometer model used to characterize the response vectors of primary utricular afferents and utricle-sensitive central vestibular neurons (see Methods). Specifically, one-dimensional units show cosinusoidally-tuned responses to linear accelerations in the head-horizontal plane, and their responses to a rotating linear acceleration vector in the head-horizontal plane are linearly related to responses to linear acceleration stimuli produced by whole-body translations or sinusoidal tilts. One consequence of this linear, one-dimensional character is that we can use responses to bidirectional OVAR to characterize the orientation, gain, and phase of unit responses to linear accelerations to linear acceleration stimuli more generally. In particular, results from responses of one-dimensional units to OVAR provide a basis for investigating the hypotheses originally generated from unit responses to position trapezoids.

Hypothesis 1: PB units are sensitive to linear accelerations in the head-horizontal plane. Existing data from whole-body position-trapezoid stimulation (Balaban et al., 2002) suggested the

presence of linear acceleration sensitivity in PB units, based on responses to brief static tilts. Constant velocity OVAR produces a rotating linear acceleration vector in the head-horizontal plane. Responses to rotating linear acceleration vectors have been used to characterize the orientation, gain, and phase of unit responses to horizontal linear acceleration (Loe et al., 1973; Schor et al., 1984; Schor et al., 1985; Manzoni et al., 1995). We predicted that PB units would show a per-rotational modulation in firing rate during OVAR, at the same frequency as the stimulus, indicative of linear acceleration sensitivity.

Hypothesis 2: The distribution of best response orientations in PB units are similar to those found in the vestibular nuclei. In primary vestibular afferents and in the vestibular nuclei, most studies of linear acceleration sensitivity have found that the largest number of units have best response orientations near ipsilateral ear-down tilt, with fewer units showing best response orientations near contralateral ear-down tilt and nose-down or occiput-down tilts (Adrian, 1943; Fujita et al., 1968; Curthoys & Markham, 1971; Peterson, 1967, 1970; Loe et al., 1973; Fernandez & Goldberg, 1976). We expected PB units to conform to this general property of units in the vestibular brainstem and for the largest number of linear-acceleration sensitive PB units to show best response orientations near ipsilateral ear-down tilt.

Hypothesis 3: PB units show responses consistent with convergence of vestibular inputs. Responses to OVAR in vestibular nucleus units which show a perstimulus modulation in firing rate which is dependent on the direction of rotation (CW or CCW), and 'bias' type responses to OVAR (consisting of increases or decreases in perstimulus unit firing in the absence of firing rate modulation at the stimulus frequency) have been attributed to convergence of linear-acceleration sensitive inputs with different spatial and temporal response properties on single

central vestibular units (Baker et al., 1984; Kasper et al, 1988; Angelaki, 1992a,b,c; Manzoni et al., 1995). Such responses are rarely found in primary vestibular afferents, but are common in second- and higher-order units receiving vestibular inputs. Because PB is known to be a target of efferent fibers from the vestibular nuclei, but is not known to receive primary vestibular afferents, we expected that a portion of recorded units would demonstrate responses that were generally consistent with responses of vestibular nuclear and other central motion-sensitive units receiving convergent inputs.

2.4. Surgical procedures:

All surgical procedures were conducted under aseptic conditions in an animal surgical suite at the Central Animal Facility of the University of Pittsburgh. Two female and one male macaque monkeys (*Macaca nemestrina*) were premedicated with atropine (0.05 mg/kg im) and ketamine (12 mg/kg im). After endotracheal intubation, anesthesia was maintained by inhalation of a 2–3% isofluorane-nitrous oxide-oxygen mixture. Three dental acrylic lugs were affixed to the skull for secure but painless head stabilization during recording sessions. One lug, positioned on the top of the skull, served as a pedestal for electrical connectors; the other two lugs were positioned behind the ears. At the site of the anterior lug, a 15-20mm patch of skin and periosteum was removed, and small holes were drilled in the skull with a dental burr. Small stainless steel screws were tapped into these holes, and the lug was constructed by applying layers of dental acrylic around the screws to a height of approximately 9mm. The two posterior lugs were similarly applied excepting that they were continuous with the acrylic base securing the recording chamber (below).

A search coil was implanted on the right eye to monitor eye movements, based on the technique of Judge et al. (1980). The conjunctiva was cut at the limbus, and a preformed 16mm diameter coil (3 turns of Teflon-insulated stainless steel wire) was sutured to the sclera. Lead wires were passed subcutaneously to a connector on top of the skull. The conjunctiva was sutured with 7-0 vicryl to cover the coil.[1]

A 20mm-diam, 10mm-high stainless steel recording chamber was implanted over a hole trephined in the parietal bone to permit the chamber to contact the intact dura mater. The chamber was oriented toward a target centered 1mm left of the midline and +1mm anterior to the ear bars, but angled 15° posterior from vertical. This approach permitted exploration of the left parabrachial nucleus. Stainless steel screws were anchored within the surrounding bone through small burr holes, and dental acrylic applied to fix the chamber to the skull. The chamber was filled with antibiotic ointment and covered with a tightly fitting cap.

Monkeys were adapted to entering the primate chair and remaining in the chair for <1hr/day prior to the initial surgery. Monkeys were given buprenorphine (0.1mg.kg) during the first post-surgical day and antibiotics (Chloramphenicol 40mg/kg B.I.D.) were administered prophylactically for three days. Two weeks after surgery, animals were re-adapted to the primate chair, head fixation, and remaining in the chair 2-3hrs/day while in the apparatus for vestibular stimulation. Monkeys were not trained to perform a particular task, and therefore were not food-deprived. A device for delivery of fruit juice is attached to the primate chair for use during

[1] Implanted eye coils were used primarily to monitor eye movements during exploration of the borders of PBN (which is bordered by regions with prominent eye-movement related responses, as noted below), for online monitoring of the altertness of the animals and to allow the monitoring of unit responses relative to eye movements. However, in this experiment and the previous experiments, no relationships between spontaneous eye movements and unit activity were observed. In the previous experiment (Balaban et al., 2002) no eye movement related responses were observed despite deliberate examination of eye movements and unit firing during smooth pursuit (C.D. Balaban, personal communication). Therefore no experimental manipulations necessary to examine eye movements in detail were performed. However, this subject is discussed briefly in **Chapter 3**.

experiments. Food preferences were assessed during the training period and animals were provided their preferred foods whenever possible.

2.5. Extracellular recordings:

Extracellular single-unit recordings were obtained with 0.005" tungsten electrodes (Micro Probe, Potomac, MD) which were positioned with a Trent Wells X-Y stage attached to the implanted chamber, and a Trent-Wells hydraulic microdrive. A 60-75mm stainless steel guide tube protected the electrode as it was lowered through the dura and tentorium cerebelli. Signals were amplified conventionally, and filtered to remove 60Hz noise. Unit and chair position data were recorded to digital tape (Cygnus CDAT16) at 24kHz for off-line analysis.

To positively locate PB units, the left abducens nucleus and left trochlear nerve root were identified as landmarks by noting their characteristic burst-tonic properties during eye movements (Fuchs and Luschei 1970). The most useful landmark in locating the parabrachial nucleus is the abducens nucleus, which contains densely packed cells with 'burst-tonic' activity related to spontaneous eye movements. These cells exhibit a high-frequency burst of spikes during saccades directed toward the side of the recording and a tonic firing rate related to eye position (Fuchs et al., 1988; Fuchs & Luschei, 1970). A second major landmark is the trochlear nerve, which lies along the rostral margin of the rotation-sensitive sites in PB. These fibers are easily recognized by their characteristic eye-movement related (down burst-tonic) discharges (Fuchs & Luschei, 1970). Such responses were evident by direct observation of eye movements during recording. The parabrachial complex also lies dorsal and lateral to the mesencephalic nucleus of the trigeminal nerve, which contains cells that discharge phasically during jaw movements while monkeys are eating or drinking. Such units are frequently encountered during recordings when the electrode has passed through the PB, and they emit a characteristic multi-

unit, bursting 'chattering' during mouth movements and typically also when the animal's cheek is brushed or indented with a cotton swab. The parabrachial complex also surrounds and is interdigitated with the superior cerebellar peduncle, which appears as an area of very sparse activity using our current electrodes. Finally, as the PB lies immediately rostral to the vestibular nuclei, the known response properties of vestibular nucleus neurons during rotation and spontaneous eye-movements were used to help determine the borders of PB.

Single units were separated from background noise using an amplitude-based window discriminator with optional 60Hz and spike width filters, written in LabView (National Instruments). Spike times were identified to 0.1-ms precision.

In order to prevent aliasing from instantaneous rate measures in datasets with few points, spike rates for all responses were represented at each spike time as the average of the two instantaneous firing rate measures (i.e. the reciprocal of the interspike interval)) calculated with respect to the preceding and following spikes.

2.5.1. Recording sessions:

Recording sessions began after a 2-wk recovery period. All experiments were conducted in the dark. Animals were placed in a five-axis rotation device (described above) in a soundproof and shielded booth. The monkey was seated in a primate chair with its head fixed to the chair in the stereotaxic plane. Recording sessions lasted for 3-4 hours each day. Animals were alert during all experiments. Stimuli presented in each session are discussed below.

2.5.2. Stimulus delivery

These experiments used a five-axis vestibular stimulator (Neuro-Kinetics, Pittsburgh, PA). The major axis is capable of rotating the entire device either periodically or at constant-velocity about

the vertical (yaw) axis. Nested in this 'outer yaw' axis is a single pitch/roll axis, capable of rotating the inner frame about a horizontal axis. Within the inner frame is a primate chair, mounted on two additional axes: the first is an 'inner yaw' axis, used to reorient the interaural axis of the animal relative to the pitch/roll axis; the second is able to reposition the primate chair vertically, in order to center the animal's head about the pitch/roll axis. The entire assembly is mounted on a driven tilt axis, which can reorient the vertical axis of the entire assembly relative to earth-vertical.

Each axis described above is capable of periodic motion within a wide range of amplitudes, with arbitrary motion profiles. In addition, the Outer Yaw Axis is capable of continuous, constant-velocity rotation. The tilt, inner yaw, and vertical linear axes are used primarily to reposition the animal prior to imposing periodic and/or continuous rotations about the pitch/roll or outer yaw axes, which are the primary driven axes for stimulus delivery.

Single units were identified initially in the darkened booth using a search stimulus of 0.7Hz sinusoidal oscillation in the pitch plane (±10-20° amplitude) with simultaneous 0.7Hz sinusoidal oscillation about the yaw axis. Note that this compound stimulus also provides a component of roll stimulation. Units showing any modulation with the search stimulus were tested with OVAR stimuli

Units showing any modulation with the search stimulus were tested with position trapezoid stimuli in the darkened booth in the following planes: *1*) pitch plane; *2*) an approximate left anterior-right posterior semicircular canal plane (LARP, animal rotated -45° (CW) from the pitch plane); *3*) an approximate right anterior-left posterior semicircular canal plane (RALP, animal rotated 45° (CCW) from pitch); *4*) roll plane (animal rotated -90° (CW) from pitch); *5*) yaw plane with head level. The position trapezoid stimulus can be described as being composed

of four separate segments: two static position segments (S1 and S2), and two transient rotation periods (T1 and T2), as shown in **Figure 2.1**. The angular displacement about the central position was ±9-15° for animals L and K, and ±20° for animal T, peak angular velocities 80 or 240°/s respectively; the duration of static position periods (S1 and S2) was 1.5s or 2.0s; the duration of transient periods (T1 and T2) was approximately 0.25s for animals L and K, and 0.5s for animal T.

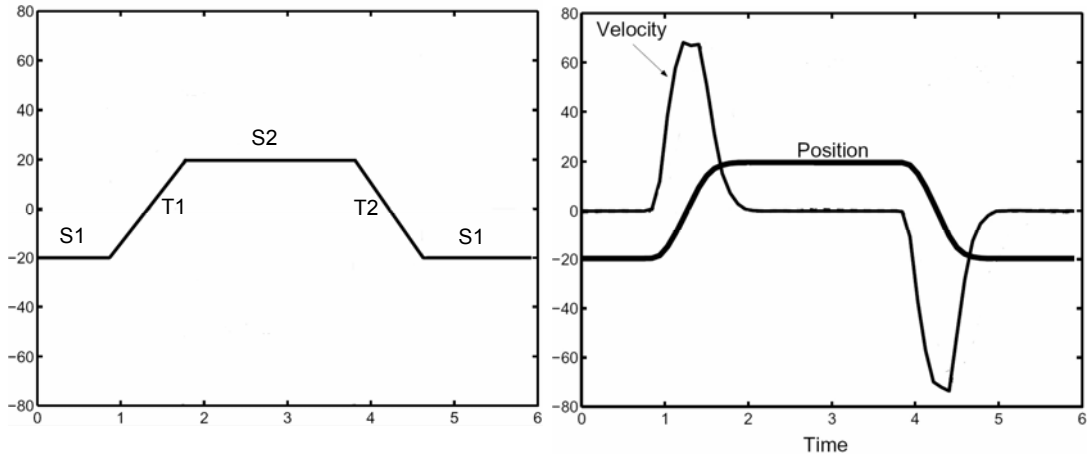

Figure 2.1 Schematic and actual position trapezoid stimulus profiles

Left panel: the idealized position trapezoid stimulus, illustrating the components as two static position periods (S1 and S2) and two transient rotation periods (T1 and T2). *Right panel*: actual position trapezoid stimulus profile used in recordings from animal T. The light trace shows the angular velocity stimulus resulting from the transient rotations at T1 and T2. See also **Figure 1.3**.

Following testing with position trapezoids, responses to OVAR stimuli were collected. The OVAR stimulus was delivered by first tilting the animal's yaw axis 15 degrees away from the earth-vertical axis, then rotating at constant velocity about the tilted axis. This stimulus is discussed further below (see Data Analysis). For animals L and K, the velocities tested were 25, 50, and 100°/s (equal to frequencies of 0.07Hz, 0.14Hz, and 0.3Hz). Due to changes in experimental equipment, for animal T the velocities tested were 20, 40, and 80°/s (equal to 0.06Hz, 0.1Hz, and 0.2Hz).

2.6. Data analysis:

2.6.1. Position Trapezoid Data:

Data were analyzed according to the method described by Balaban (Balaban et al., 2002). However, that article used a 'primary afferent transformed' representation of angular velocity as input to the model used to fit unit responses. Rather than this modified velocity model, the untransformed angular velocity is used here. Other criticisms of the stimulus and analysis used by Balaban et al. (2002) are addressed in the discussion and in **Chapter 1**.

Whole-body vertical position data were obtained directly from the vertical plane rotation device, and differentiation of this signal yielded a vertical velocity signal. Models used to fit unit responses to vertical position trapezoids contained parameters for angular position, angular velocity, and leaky integration of angular position, as necessary to account for the responses of individual units. The parameters used in this model were 1) whole-body vertical position (tilt), 2) whole-body angular velocity, and 3) a leaky integration of angular velocity (Balaban et al., 2002). The latter is differentiated from position (i.e., the integral of velocity) and angular velocity by the inclusion of integration and leakage time constants. The leaky integration of velocity (illustrated in **Figure 1.4**) is defined as $v_i = R_{int} - R_{leak}$, where R_{int} is integrated angular head velocity with transfer function:

$$\frac{1}{0.2s}$$

and R_{leak} is the result of inputting R_{int} to a first order low pass filter with transfer function:

$$\frac{1}{0.25s+1}$$

Note that the above equation for v_i is approximately equivalent to a high pass filtered representation of vertical angular position, of form

$$\frac{s}{0.2s+1},$$

however, no combination of coefficients in this transfer function could precisely replicate the timecourse and magnitude of the 'leaky integrator' model.

Because the responses of most units to velocity transients were asymmetric with respect to the direction of rotation in some or all parameters, separate gain parameters were estimated for upward and downward velocity and leaky integrated angular velocity sensitivities.

The complete response model just described is:

$$R_T = b + (g_{pos} * H) + (g_{up-vel} * \max(0, H')) + (g_{up-v_i} * L_{up}(H')) +$$
$$\ldots (g_{down-vel} * \min(0, H')) + (g_{down-v_i} * L_{down}(H'))$$

where R_T is the total response of the cell, b is baseline firing rate, g_{pos} is vertical angular position gain, H is vertical angular head position (i.e. angle of whole-body tilt), H' is whole-body angular velocity (Balaban et al. 2002), $g_{up\text{-}vel}$ is velocity response gain for upward rotation (i.e. nose up when the chair is positioned in the pitch plane), g_{up-v_i} is the gain for upward leaky integrated angular velocity, and L_{up} is the leaky integrator described above, applied to the upward velocity transients. Separate gain parameters were estimated for oppositely-directed rotations because of obvious asymmetries in unit responses to angular rotations, which have also been noted in vestibular nucleus unit responses (see, e.g., Peterson, 1970; Curthoys & Markham, 1971). As with OVAR data, subsets of this model describing unit behavior without position sensitivity or without both position and integrated velocity sensitivity were fit to the data and compared using the standard F-test for nested models (see **2.6.2**, below). Coefficients for more complex versions of the model are reported only when they provided a significantly better fit to the data than all simpler models (α=0.01). As in the prior report, the model was sufficient to account for all observed response patterns. Responses to yaw position trapezoids were similarly modeled, with the omission of the position-sensitivity parameter.

2.6.2. Responses to single OVAR stimulus presentations:

The OVAR stimulus and the use of responses to multiple OVAR stimulus conditions to estimate the response properties of PB units to linear acceleration is discussed in the following sections. In order to use OVAR data to determine the parameters describing responses of units to linear acceleration generally, it is first necessary to describe responses to multiple OVAR stimuli individually. Specifically, data from at least one stimulus frequency (or velocity) in both the CW and CCW directions of rotation is necessary. Therefore we must first obtain intermediate parameters representing responses to individual OVAR stimuli, in the following manner.

Data from each OVAR stimulus cycle was divided into 64 equally-spaced bins, and average instantaneous firing rates in each bin were used to compute firing rates across an average stimulus cycle. Yaw-axis position data were obtained directly from the position feedback signals for the rotation device. Tilt angles for OVAR were obtained directly from an inclinometer mounted to the device.

Responses of units to individual OVAR stimulus conditions were described as background firing rate plus (for units with significant modulation responses) modulation gain and the table position at which the peak-response appeared, relative to the nose-down orientation. Nonlinear least-squares regression (Levenberg-Marquardt algorithm as provided in MatLab 6.1 (The Mathworks, Inc., Natick, MA)) was used to estimate unit responses during individual OVAR conditions as:

$$R = \max(0, \mu + \rho \cos(\theta_0 - \delta))$$

where R is the total response of the cell, μ is the background (mean) firing rate, ρ is the gain of the modulation component of the response (equal to one-half of the peak-to peak modulation amplitude), θ_0 is the initial table position (at nose-down tilt), and δ is the peak response direction

relative to nose-down. All models take the maximum of the fitted model or zero at each timepoint, as neurons cannot fire at negative rates (see, e.g. Melvill Jones & Milsum, 1970; Fernandez & Goldberg, 1976c). The use of this data to determine linear acceleration response characteristics is described below.

The data were also fit with a line representing the average firing rate over the entire stimulus presentation. A nested F-test was used to determine whether the cosinusoidal fit accounted for significantly more variance than the simple average rate fit. The F-test for comparing two nested models, (one of which is a simplified version of, and has fewer free parameters than the other), is:

$$F = \frac{(SSR1 - SSR2) \big/ SSR2}{(DF1 - DF2) \big/ DF2}$$

where SSR2 is the sum of squared residuals of the more complex model, and the degrees of freedom are the number of data points minus the number of free parameters in each equation: *DF*=((*number of datapoints*) - (*number of free model parameters*)).

Because

$$\text{variance} = s^2 = \frac{SSR}{DF},$$

we can rewrite the previous equation as:

$$F = \frac{s^2_{\text{total}} - s^2_{\text{model}}}{s^2_{\text{model}}}.$$

to illustrate that the nested F-test provides a measure of whether the increase in variance accounted for by the more complex model (in this case, the cosine model) is significantly greater than the total variance of firing rate about the less complex model (in this case, the mean). If the simpler model is correct, we would expect an F value near 1.0. A larger value of F means that the increase in sum-of-squares when we go from the more complicated model to the simpler model

is greater than the proportional increase in the degrees of freedom. If we get an F value much greater than 1, there are two possible explanations: 1) the more complicated model is correct, or 2) random scatter in the data led to a better fit by the more complicated model. The *p*-value provided by the standard F tables tells us how rare the result would be if the latter explanation is correct. The only requirements of the nested F test are that the errors in the data be approximately normally distributed, and that both models are fit to the same data. This is true for our data; however, to allow for minor deviations from normality we have further biased ourselves against Type I, or false-positive, errors by adopting $\alpha=0.01$ for this test. While the choice of statistic for testing whether or not there is a significant modulation at the frequency of the stimulus is inherently subjective[2], the appropriateness of this test is supported by the success in distinguishing *modulated* and *unmodulated* units which show other distinct properties, as discussed in **Results**, below.

Responses for which the cosinusoidal fit did not account for significantly more variance than the mean (by comparing F to $F_{crit(DF1-DF2,DF2)}$ using standard F tables for $\alpha=0.01$), were

[l]Other studies (see, e.g., papers by Golberg, Fernandez, Schor, Angelaki and others on responses to sinusoidal stimuli) have used Fourier analysis to determine the relative powers of the fundamental versus higher frequencies in the recorded data (to determine whether firing rate modulation at the frequency of the stimulus is significant and/or greater than the total remaining spectral power contained in the unit response). However, evaluation of the results of Fourier analyses and of exact tests of significance on relative powers of particular frequencies in a signal are cumbersome (approachable discussions of tests of significance in harmonic analysis of time-series data have been given by Hartley (1949), and Shumway (1971)), and most authors have adopted 'rules of thumb,' such as requiring that the power of the fundamental be greater than 1.5 times the power of frequencies above the 2nd harmonic (Schor & Miller, 1982). In addition, the Fourier analysis breaks down when modulated responses are 'clipped' at 0sp/s, and in such cases previous authors have reverted to common nonlinear regression approaches to estimate the gain and phase of unit responses to sinusoidal stimuli by fitting clipped sinusoids to responses (Melvill Jones & Milsum, 1970; Fernandez & Goldberg, 1976c). For the purposes of this document, the nested-F test is more readily interpretable. However, we can use Parseval's theorem to show the correspondence between the two approaches. Parseval's theorem states that the sum (or integral) of the square of a function (i.e. the total sum of squares in the data) is equal to the sum (or integral) of the square of its transform (e.g. the Fourier transform). That is, the sum of squares of the powers of all frequencies in the Fourier-transformed data is equal to the total sum of squared differences from the mean in the untransformed data. When applied to a comparison of the cosine fit to the data (with the cosine frequency equal to the fundamental frequency of the stimulus) versus the mean firing rate of the data, the nested F-test therefore answers the question of whether the magnitude of response variation at the fundamental frequency contained in the data is significantly greater than the total remaining spectral power contained in the data, with the additional advantages of simplicity and of being able to assign a specific probability to the result.

considered unmodulated. Accordingly, gain and peak response direction parameters from the above fit were included in analyses only when the cosine fit accounted for significantly more variance than the mean firing rate model, and were otherwise set to zero.

2.6.3. Characterization of linear acceleration sensitivity from unit responses to OVAR:

Primary vestibular afferents innervating hair cells in the utricular macula respond maximally to forces acting in a plane which is inclined rostrally approximately 20-30º from the head-horizontal plane, depending on the species, and is parallel to the plane of the utricle (Loe et al., 1973). Responses of primary utricular afferents and many central vestibular neurons conform to a response model which treats these units as one-dimensional linear accelerometers. The responses of one-dimensional units to a static linear acceleration stimulus can be defined by reference to an 'orientation vector,' ξ, (Schor et al., 1998) or 'polarization vector' (Loe et al, 1973), which is simply a unit vector specifying the head-referenced direction of linear acceleration eliciting a maximal response, and a gain parameter, ρ, which specifies the sensitivity of the unit to stimulation in this direction (Fernandez et al., 1972). These units have responses to head-horizontal plane linear accelerations which are linearly related to the component of the applied force acting parallel to the unit's orientation vector (Lowenstein & Roberts, 1950; Fernandez et al., 1972). A consequence of one-dimensionality is that, if we apply a force in a direction that is not parallel to the unit's orientation vector, we can decompose the applied force into a component which is parallel to the orientation vector, and a force directed orthogonally to it. The net force acting along the peak response vector of the unit is then given by $F_{net} = F_{applied}\cos(\theta)$, the so-called 'cosine rule' (Fernandez et al., 1972; Fernandez & Goldberg, 1976; Angelaki, 1991; Schor & Angelaki, 1992). The force component which is perpendicular to the unit's orientation vector has no influence on unit activity (**Figure 2.2**).

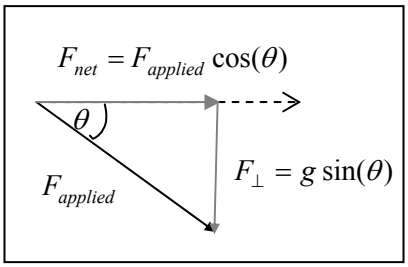

Figure 2.2 Response to linear acceleration in the head-horizontal plane.

Components of force acting parallel and perpendicular to an orientation vector (*dashed arrow*), by an applied force, $F_{applied}$.

Similarly, gravity imposes a constant linear acceleration along axes perpendicular to the earth-horizontal plane. With the head upright, the gravity vector is, to a first approximation, orthogonal to the plane of the utricle and exerts no net force in the plane of utricular hair cells' sensitivity. If the head is tilted with respect to gravity, we can decompose the forces acting on the utricle into a force acting parallel to the plane of the utricle, and a normal force, acting perpendicular to the inclined plane (**Figure 2.3**). The net force acting in the plane of the utricle is given by $F_{net} = g\cos(\theta)$, where g is the acceleration due to gravity, and θ is the angle between the gravity vector and the plane of the utricle. The normal force has no influence on cell activity.

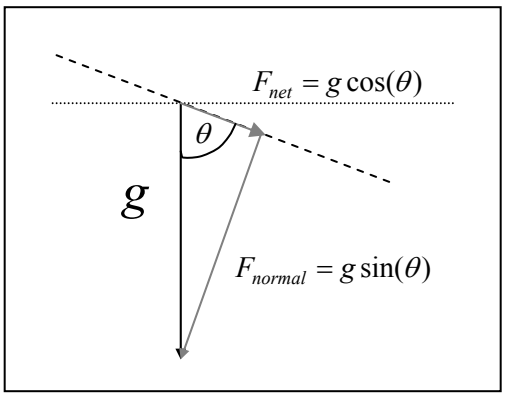

Figure 2.3 Linear acceleration force components due to gravity

If the head is tilted with respect to gravity, the net force acting in the plane of the utricle is proportional to the angle of tilt. *g*: gravity vector. *Dotted line*: Earth-horizontal. *Dashed line*: plane of utricle.

Combining the above, we can treat the stimulus acting in the plane of the utricle (**Figure 2.3**) as an applied force vector with an orientation defined by the intersection of the plane of the utricle

and the vertical plane of tilt. If the direction of tilt is not parallel to the orientation vector of a linear acceleration sensitive unit, the net force acting along the orientation vector is again related to the angle between the applied force vector and the orientation vector, as shown in **Figure 2.2**.

2.6.4. OVAR stimulation:

If we first tilt the animal with respect to gravity, then rotate about that tilted axis at a constant velocity, we impose a linear acceleration vector, due to gravity, on the plane of the utricle, which rotates about the head in the opposite direction to turntable rotation. For a one-dimensional linear accelerometer, this stimulus is physically equivalent to the rotating linear acceleration vector produced by simultaneous cosinusoidal tilt about the pitch and sinusoidal tilt about the roll axis—the "wobble" stimulus used by other authors in canal-plugged animals or for recordings in primary afferents (Schor et al., 1984; Schor et al., 1985; Manzoni et al., 1995). Thus, 40º/s CW OVAR, with the rotation axis tilted 15º away from the earth-vertical produces a linear acceleration stimulus of magnitude $\cos(75º)*(9.8m/s^2)=0.26g=2.54m/s^2$ (**Figure 2.3**), rotating CCW about the head at 40º/s. Such "off-vertical axis rotation" stimuli may be used to characterize responses to linear accelerations in the plane of the utricle (Manzoni et al., 1995; Schor et al., 1998). At low angular velocities, the centrifugal force due to rotation is negligible, and may consider only the effective force due to gravity acting in the plane of the utricle (**Figure 2.3**) and in the vertical plane of tilt. A consequence of the linearity assumption is that the response of the unit to a cosinusoidally-varying force acting along a unit's orientation vector also varies cosinusoidally with time, at the same frequency as the imposed force. A consequence of one-dimensionality is that the response is dependent only on the angle between the applied force vector and the orientation vector (**Figure 2.2**) Then, regardless of the direction of rotation, the

effective force acting in the direction of the orientation vector is modulated identically for a given stimulus magnitude and frequency. In particular, for linear, one-dimensional units, response gains during CW rotation should be the same as gains during CCW rotation. We will show (see **Results**) that for most of our units, bidirectional modulation gains were indeed the same.

Response phase and orientation:

Responses to a time-varying, periodic stimulus require the introduction of a third parameter (response phase, φ) to describe the temporal, frequency-dependent relationship between the peak of the imposed force acting along the orientation vector and the location of occurrence of the peak response in the stimulus cycle (**Figure 2.4**). Peak responses to time-varying stimuli in the vestibular system routinely lead or lag the presentation of the peak stimulus (Schor et al., 1984, 1985; Schor & Angelaki, 1992; Manzoni et al., 1995; Angelaki, 1992b). Temporal differences between stimulus application and unit response may be introduced by active or passive processes in the central network processing the stimulus, or by intrinsic properties of the sensory apparatus. For linear, one-dimensional units, phase is constant for linear-acceleration stimuli applied in all directions, but may vary with stimulus frequency (Schor et al., 1985). Gain may also vary with stimulus frequency, but response amplitude is a function of stimulus direction, as noted above. For a cosinusoidal linear acceleration stimulus produced by translation along a linear track or by sinusoidal rotation in vertical planes, the stimulus orientation producing the greatest response is defined unambiguously. However, if the applied stimulus *direction* varies with time, the apparent location of the peak response is a function of stimulus phase and will result an apparent shift in response direction relative to stimulus direction. Therefore we must use bidirectional OVAR rotation at each frequency tested in order to uniquely determine the orientation and phase

parameters, while either direction of rotation gives an unambiguous estimate of response gain. Responses to bidirectional OVAR then allow us to find the three-vector of parameters which characterize the response of a unit to a time-varying linear acceleration stimulus: (ξ, ρ, ϕ).

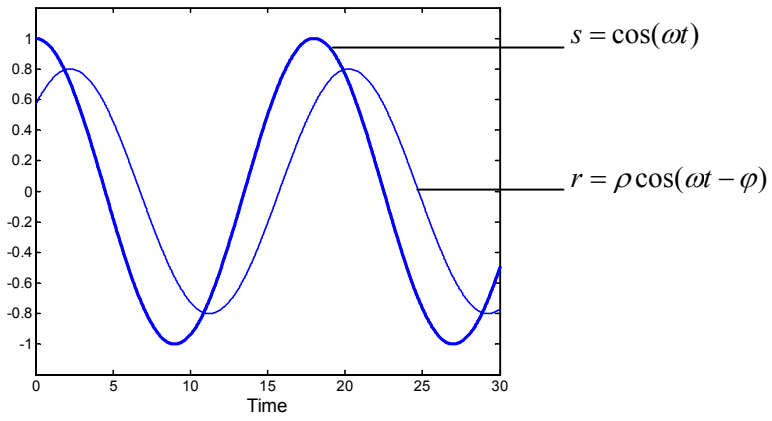

Figure 2.4 Response to cosinusoidal stimulus

Depiction of the effect of response gain and phase on peak response location within a stimulus cycle. The applied force varies cosinusoidally along the response vector at 0.056Hz (equivalent to OVAR rotation at 20°/s). The response has a gain of ρ=0.8 and a phase lag of φ=45°. The stimulus varies as $\cos(\omega t)$, resulting in a response which varies as $0.8 \cdot \cos(\omega t - 45)$.

Following the above argument, there exists a correspondence between stimulus magnitude along the orientation vector, and table position. Then, during rotation, the change in stimulus magnitude along the orientation vector with time is proportional to the change in table position with time. Working backwards, the difference between the table position at which the response peak occurs and the table position representing the direction of tilt corresponding to the unit's orientation vector provides the estimate of φ, the unit's response phase. The response phase remains fixed relative to the temporal modulation of stimulus magnitude acting along the orientation vector, but the stimulus *direction* at which the response peak occurs is now dependent on the direction of rotation of the table, with:

$$peak_{CW} = \xi - \varphi$$
$$peak_{CCW} = \xi + \varphi \text{,}$$

and these equations can be solved to give:

$$\xi = \frac{peak_{CW} + peak_{CCW}}{2}$$
$$\varphi = \frac{peak_{CCW} - peak_{CW}}{2}$$

All calculations were confirmed by inspection, as shown in **Figure 2.9**. Note that in describing unit response vectors, we have arbitrarily assigned the orientation vector to the acute angle described by the peak response directions during CW and CCW rotation at the lowest stimulus frequency for which responses were available for each unit. Then, small phase *leads* as determined by this arbitrary assignment are equivalent to large phase *lags* with the orientation vector pointing in the opposite direction. It is not possible to unambiguously determine the 'true' peak excitatory response direction from OVAR stimulation alone. Rather, we would need to use longer-duration linear acceleration stimuli to do so (see e.g. Fernandez & Goldberg, 1976b)

2.7. RESULTS

2.7.1. Available data:

Data from vertical-plane position trapezoid stimulation were available for 101 units. Data from yaw-axis position trapezoids were available for 80 of these units. The number of units for which OVAR data were available are given in **Table 2.1**.

	OVAR Velocity					
Animal:	20º/s	25º/s	40º/s	50º/s	80º/s	100º/s
L		5		5		
K		19		48		58
T	12		25		21	
Totals:	12	24	25	53	21	58

Table 2.1 Bidirectional OVAR data available from each animal

Table gives the number of units for which data was available from both CW and CCW rotation at each angular velocity. A total of 103 units were tested with bidirectional stimulation at at least one frequency. The change in stimulus velocities used for animal T was due to equipment changes. The OVAR velocities given in the table are equivalent to rotation *frequencies* of approximately 0.06, 0.07, 0.1, 0.14, 0.2, and 0.3 Hz.

2.7.2. Responses to position trapezoid stimulation:

As mentioned in the introduction, we have first set out to replicate the basic findings of Balaban et al (2002), prior to moving on to the description of responses to OVAR. This section presents sufficient examples of unit responses and descriptions of aggregate response characteristics to demonstrate that we have reproduced the complex responses described in the previous studies. In many examples, we have provided illustrations of unit response which are more clear than those provided in the earlier study. This improvement in apparent response characteristics is due in part to the use (in animal T) of slightly longer-duration position trapezoids, and use of a table capable of more controlled motion profiles, which introduces less noise (e.g. mechanical and control loop 'ringing') into the input signal and thus the response as well. However, we have already admitted in the introduction and throughout this document that it is quite difficult to interpret responses to position trapezoid stimuli beyond describing their responses with a model which provides a good fit to the data, despite its own interpretational hurdles regarding confidence in the relative magnitudes assigned to each response component in a given fit (see **Chapter 1**). However, the presentation of results given here will be brief and only sufficient to demonstrate correspondence between the basic properties of position trapezoid responses reported by Balaban et al. (2002), and trapezoid responses in the current data set. We will then show (**Section 2.7.5**) a few simple comparisons with OVAR responses to illustrate the difficulties inherent in attempting to draw any specific conclusions from trapezoid responses and the necessity to select more readily interpretable stimuli (e.g. stimuli such as OVAR which produce some minimal degree of internal consistency in unit responses and analysis procedures) for future studies.

Figure 2.5 shows the responses of several units to vertical plane position trapezoid stimuli in one or more planes. Each plot represents the average unit response over 10 or more stimulus cycles. These plots illustrate the variety of unit responses and fits given by the model discussed in Methods. Two responses with prominent components of sensitivity to angular velocity are depicted in panels *d* and *e*. Both panels show responses to trapezoidal tilts in the RALP plane, in different units. The unit in panel *d* and *g* has an unrectified response to angular velocity in both RALP and LARP planes, while the unit in panel *e* has a positively rectified response to velocity. Inspection of the response and comparison to the model components depicted in the lower left figure shows the need for the inclusion of the leaky integrator component.

The models describing unit responses shown in plots *f*, *h*, and *i* had prominent integrated angular velocity sensitivity coefficients. In plot *h*, the velocity component of the stimulus (dashed line) is also plotted, illustrating the difference between the response peak and the velocity transient. The response in figure *f* shows a correspondence between "integrated angular velocity" response gain and static tilt response gain. This plot demonstrates the difficulty in distinguishing between transient responses due to angular velocity (as implied by the use of "integrated angular velocity" in the response model proposed by Balaban et al. (2002)), and transient responses due to phasic linear acceleration responses (Peterson, 1970). In this unit, (as well as the units in panels *c*, *h*, and *i*), the magnitude of the phasic component of the response was not symmetric with respect to rotation direction, however, as might be expected in linear acceleration-sensitive vestibular nuclear neurons (Peterson, 1970; Fernandez & Goldberg, 1976), but is rather very large in one direction and minimal or absent during oppositely-directed tilt. The response in panel *i*, shows a transient response to tilt in the absence of a sustained static tilt

response, suggesting that the response is not due to linear acceleration sensitivity. Note that the 'integrated angular velocity' component of the fit in panel *i* seems to underestimate the time-constant of the response decay. This illustrates one limitation of the approach with respect to the choice of a fixed value for the leaky integrator time constants.

Responses in panels *b* and *j* were modeled with angular velocity and integrated angular velocity coefficients of opposite sign (compare to **Figure 1.5**, lower right panel). To illustrate the difference in timecourse between this model and the timecourse of angular acceleration, the angular acceleration imposed by the stimulus is plotted (dashed lines).

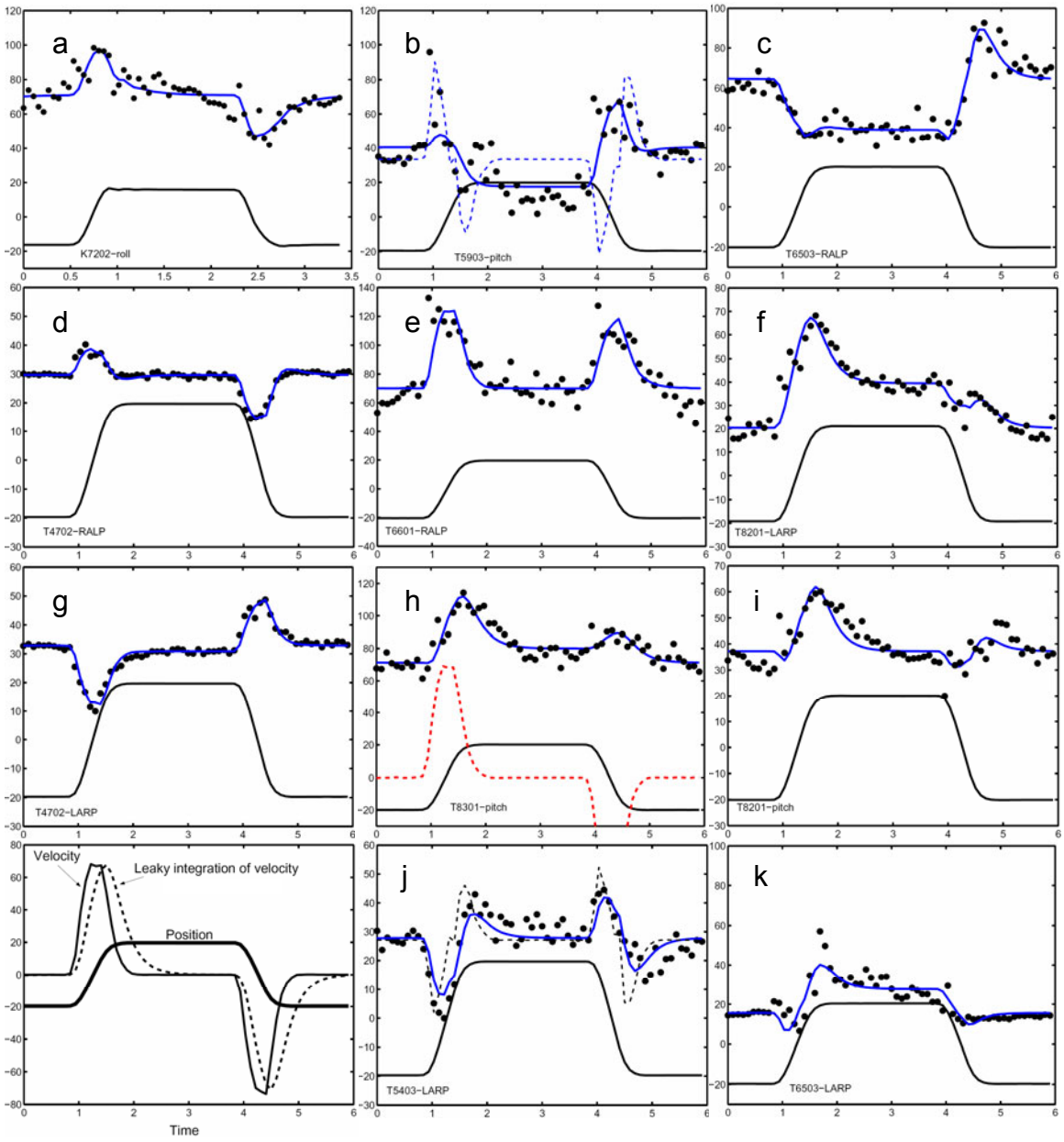

Figure 2.5 Example responses to vertical plane position trapezoids

Lower left: schematic of stimulus and response model components based on the analysis given by Balaban et al. (2002). See text for discussion of all responses shown. In all panels, the position stimulus (solid lower trace) is depicted, with the unit response (points), and model fits (solid line). *x-axis*: time; *y-axis*: firing rate in spikes per second. Plots are scaled according to unit response rates, so the amplitude of tilts appears to vary between plots.

As described by Balaban et al. (2002), parameters of model fits to unit responses to position trapezoid stimuli in the pitch, roll, LARP, and RALP vertical rotation planes, as described in

Methods, were plotted as a function of stimulus orientation and fitted with symmetric or asymmetric cosines, as shown in **Figure 2.6,** to obtain estimates of the stimulus direction producing the maximal response associated with each model component (i.e. static tilt, angular velocity, and a leaky integration of angular velocity).

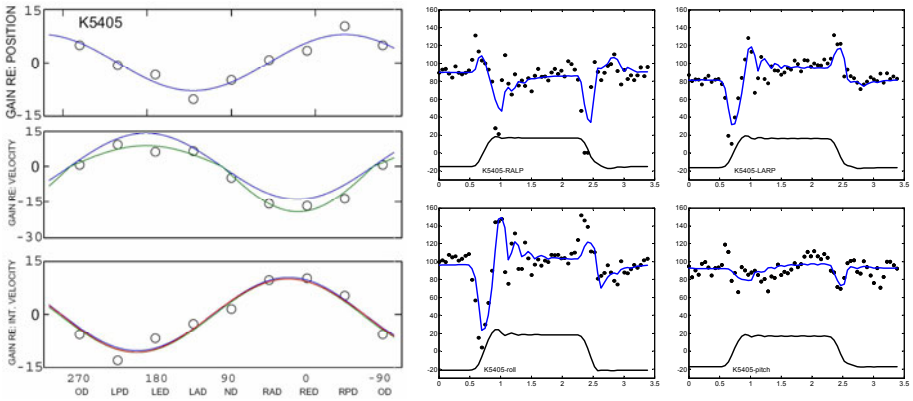

Figure 2.6 Example response to position trapezoids and response profiles

Right panel: Unit responses to trapezoidal tilts in all vertical rotation planes, noted in each plot. *Left panel*: cosine fits to the static tilt, angular velocity, and leaky integrated angular velocity parameters from the model fits show on the right. *x-axis*: stimulus orientation re: head. *y-axis*: values of gain parameters for each response component. Static tilt is in sp/s/g of linear acceleration force, and angular velocity is in spikes/s/^{o}/s.

2.7.2.1. Responses to rotation transients

Orientation of peak velocity responses

As in the previous report (Balaban et al. 2002), units were classified on the basis of the three-dimensional orientation of the rotation axis producing the peak response to angular velocity.

Responses to vertical plane (i.e. pitch, roll, and vertical canal planes) rotation transients could be rectified or unrectified with respect to rotation direction. Most units (69/87; 79%) showed unrectified responses to vertical-plane rotation velocity, with a maximum excitatory response during rotation in one direction and an inhibitory response to the opposite direction of rotation velocity. Of the units with rectified angular velocity responses in their plane of maximum sensitivity, 16/17 units had upwardly rectified (bidirectionally excitatory) angular velocity responses, with a single unit showing a downwardly rectified response, with all recorded

vertical-plane angular velocity responses being inhibitory. Units were also classified on the basis of their yaw-axis angular velocity sensitivity (Balaban et al., 2002) and the presence or absence of rectification in their angular velocity response, as follows:

Vertical-plane-only angular velocity-sensitive neurons:

Twenty-seven of the 80 units (34%) with complete vertical- and yaw-position trapezoid datasets responded to whole-body angular velocity only in vertical planes, with no yaw-velocity sensitivity (i.e. $\varphi_{vel}=0$). The velocity responses of all 25/27 of these units were unrectified for rotations in the vertical plane producing the peak response, with an excitatory response to rotation in one direction, and an inhibitory response to oppositely-directed rotations. The two exceptional units showed non-inhibitory responses (either excitatory or no response) to all vertical-plane position trapezoid transients.

Vertical-plane plus yaw angular velocity-sensitive neurons:

Fifty-four of 80 units (67.5%) tested with yaw-position trapezoids showed some sensitivity to yaw-axis velocity. Of these 54 units, 28 units (52%) showed increased firing rates during CCW yaw velocity, while 19 units (35%) showed higher firing rates during CW rotation. Rectified units (n=29; 54%) were excited or inhibited by rotation in both directions. Fourteen units with rectified responses to yaw-axis rotation transients had larger responses to CW rotation, and fifteen units preferred CCW rotation. Twenty-six rectified units had excitatory responses to both CW and CCW yaw velocity, while the remaining three units had inhibitory responses to bidirectional yaw velocity.

Integrated angular velocity sensitivity:

The tuning of the leaky integrated velocity-like components of unit responses to trapezoidal tilts in pitch, roll, and approximate vertical canal planes was estimated using an asymmetric cosine fit to responses to tilt in eight directions, which allowed for separate gains for each half of the response, so that for example, gains tuned precisely in the roll plane might have separate response gains for tilts in the left-ear-down direction and in the right-ear-down direction. In such a case, a unit with an unrectified response might have an excitatory response to left-ear-down tilt and an inhibitory response to right-ear down tilt, whereas a unit with a rectified response might have excitatory responses to tilts in both directions, and response amplitudes plotted against stimulus direction would trace a fully rectified cosine. The response profiles (across stimulus directions) of units fitted with the asymmetric cosine model separated these neurons into units with rectified (n=31) and unrectified (n=62) responses to rotation. As in the previous report, only 57 units (56%) had the same degree rectification of both velocity and integrated velocity responses. This comparison between velocity and "leaky integrated velocity" (Balaban et al., 2002) responses is of interest because it is unresolved whether this element of unit responses derives from dynamic linear or angular acceleration sensitivities, and thus whether they derive from the same or different classes of sensory inputs (canal, otolith, orthostatic, proprioceptive or other inputs). If responses to angular velocity and responses components modeled as a sensitivity to a leaky integration of angular velocity show similar behavior when examined from a number of perspectives, including the rectification of their responses, we might conclude that they are determined by the same underlying sensitivity, such as a sensitivity to angular velocity or acceleration. The fact that we find limited agreement between the estimated orientations, gains, and rectification of response components due to velocity and to leaky integrated velocity

(implying sensitivity to position transients, rather than to fixed position) implies that the angular velocity and leaky integrated angular velocity sensitivities are determined independently.

Spatial relationships between velocity and 'integrated velocity' tuning:

Due to the presence in many units of biphasic, or sharpened, responses to velocity transients during position-trapezoid stimulation, Balaban et al. (2002) proposed that opposition of angular velocity and 'integrated velocity' peak response directions might be an essential component of self-motion responses in PB. In fact, this statement and evidence for it might rather be interpreted as evidence that these response components result from a single response process, rather than being independent in either their origins or distributions.

As suggested previously, units did show a strong tendency for velocity and 'integrated velocity' peak response directions to be opposed, so as to produce a characteristic sharpening of phasic responses to velocity transients (see e.g. **Figure 2.5j**). The orientations of peak angular velocity and integrated velocity sensitivity vectors were clustered near left ear down and right ear down rotation, respectively (see **Figure 2.17**). Peak angular velocity responses for all units were centered around left-ear-down angular rotations (Rayleigh test, $p<0.001$; unweighted circular mean orientation: 167º±31º), while peak 'leaky integrated velocity' responses were centered between right ear down and right anterior canal-down rotation (circular mean ±SD: 20º±82º Rayleigh test, $p<.001$). **Figure 2.17** shows the associated response vectors for all units.

2.7.2.2. Static Position Sensitivity:

Among units tested in all vertical rotation planes, 75/100 units showed significant static tilt sensitivity in at least one vertical rotation plane. The orientations of peak static tilt responses were distributed throughout the head-horizontal plane (**Figure 2.7**). The peak sensitivities of these units averaged 24.2(±27.7)sp/s/g of linear acceleration (**Figure 2.7**).

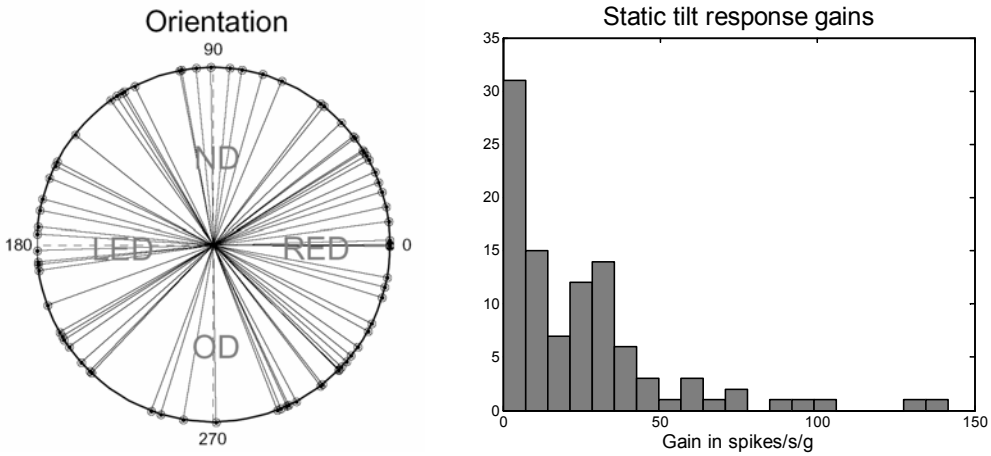

Figure 2.7 Static tilt gains from position trapezoid stimulation

Left panel: Orientation vectors of unit responses to static tilt for all units with non-zero static tilt responses (n=75). Response vectors were distributed throughout the head-horizontal plane, with no coherent orientation (Rayleigh test; $p>0.05$). Right panel: linear acceleration gains (in spikes per second per *g*) for all tested units (n=100).

2.7.3. Responses to OVAR stimulation. I. One-dimensional responses:

Following the decay of horizontal semicircular canal responses to the yaw-axis angular velocity produced at the initiation of rotation, constant-velocity OVAR produces a linear acceleration stimulus equivalent to the projection of the gravitational acceleration vector on the utricle, which rotates in the opposite direction of the rotation of the animal about a tilted axis.

For the data presented here, recordings began at least 30 seconds after the initiation of OVAR, so we assume that any angular velocity responses due to horizontal semicircular canal stimulation have decayed to an inconsequential level (Shimazu & Precht, 1965; Reisine & Raphan, 1992; Furman & Schor, 2001), and the recorded response is due to the linear-acceleration stimulus.

Estimation of the parameters describing a unit's response, (ξ, ρ, ϕ), from OVAR requires data from *both* CW and CCW OVAR at the same stimulus frequency, as described above. Therefore, for any unit, if OVAR data was available from only one direction at a particular

frequency, this data was discarded. The OVAR angular velocities used were 20, 40, 50, 80, or 100º/s, corresponding to frequencies of approximately 0.056, 0.11, 0.14, 0.22, and 0.28 Hz.

For all recorded units (n=103), the number of units tested with both CW and CCW OVAR at each angular velocity is given in **Table 2.1**, above.

OVAR response classes:

Units could be classified on the basis of the presence or absence of a significant modulation response to OVAR, at the frequency of rotation. Units with significant modulation at the stimulus frequency (using the nested F-test; see **Methods, 2.6.2**) and a modulation gain >3sp/s and a significant cosine fit with VAF>0.4, and were classified as "modulated" units. Units with insignificant modulation gain across all OVAR conditions were classified as "unmodulated." The most common unit response (65/103 units; 64%) consisted of a perstimulus modulation in firing rate during OVAR stimulation. Forty-eight of these units had properties consistent with linear, one-dimensional responses to linear acceleration during OVAR, as described below. Seventeen units had modulation responses indicative of convergence of vestibular inputs with different spatial and temporal characteristics.

The remaining 38 units showed no response (n=11), or else showed *bias*-type responses (n=17) consisting of a significant perstimulus increase or decrease in mean firing rate, in the absence of a modulation response. The following discussion focuses primarily on those units with one-dimensional responses.

2.7.3.1. Modulated responses to OVAR:

Sufficient data for analysis of responses to OVAR (defined as at least one response to OVAR in each direction at equal velocities) was available for 103 units. The most common unit response

(65/103 units; 64%) during OVAR stimulation showed a significant modulation in firing rate at the frequency of the stimulus

One-dimensional responses:

One consequence of linearity and one-dimensionality is that response gain during CW and CCW OVAR is identical (Manzoni et al., 1995; Chan et al., 1987; Chan, 1997; Angelaki, 1991, 1992b,c; Schor & Angelaki, 1992). Many central vestibular units show behavior suggesting convergence of inputs with different spatial (i.e. orientation) and temporal (i.e. phase) properties, termed spatiotemporal convergence, or STC. Responses of such units can be modeled as a sum of inputs from two units which are in spatial and temporal quadrature, with frequency dependent gains. That is, the orientations of each input unit are assumed to be 90º apart, and their phases are also assumed to be 90º apart. In this case, if we rotate the animal in the CW and CCW directions, we expect that the response gain of the STC unit will be a sum of the underlying input gains during rotation in one direction, and a difference of the input gains during rotation in the opposite direction. This can be considered to result from the difference in temporal response characteristics: if one unit has a lagging response, and the other has a leading response, then rotation in one direction at some appropriate velocity will result in a summation of the responses as they become temporally aligned, and the opposite result will obtain to rotations in the opposite direction.

We can test this assumption by taking the ratio of response gains during CW and CCW rotation. Response ratios between 0.5-2.0 (i.e. responses within a factor of two of each other) may be taken as indicating linear, one-dimensional behavior (Wilson et al., 1986; Kasper et al., 1988). Forty-eight of 65 units classified as *modulated* fit this criterion (**Figure 2.8**) and were therefore classified as one-dimensional units and analyzed as described above.

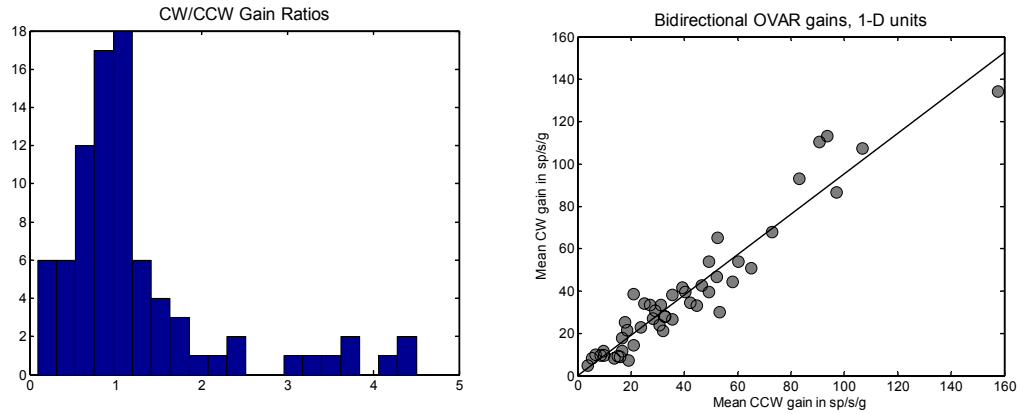

Figure 2.8 Bidirectional OVAR response gains and ratios

Left panel: Histogram of the ratio of response gains during CW and CCW OVAR for all units with modulated firing rates during OVAR. The majority of units (48) had response ratios between 0.5:2.0. The ratios here are mean ratios for all OVAR frequencies for which both CW and CCW data were available for each unit. If a unit had a response ratio <0.5 or >2.0 at *any* OVAR frequency, that unit was excluded from analyses of one-dimensional units. *Right panel*: For the 48 one-dimensional units, bidirectional response amplitudes (in sp/s at 15º tilt) were highly correlated (Pearson's product-moment correlation: 0.954, p<.001).

Linear acceleration response vector orientations:

The orientation, gain, and phase of linear, one-dimensional responses to OVAR were calculated as given in Methods. Two examples of one-dimensional responses and the calculation of response orientation and phase are given in **Figure 2.9**.

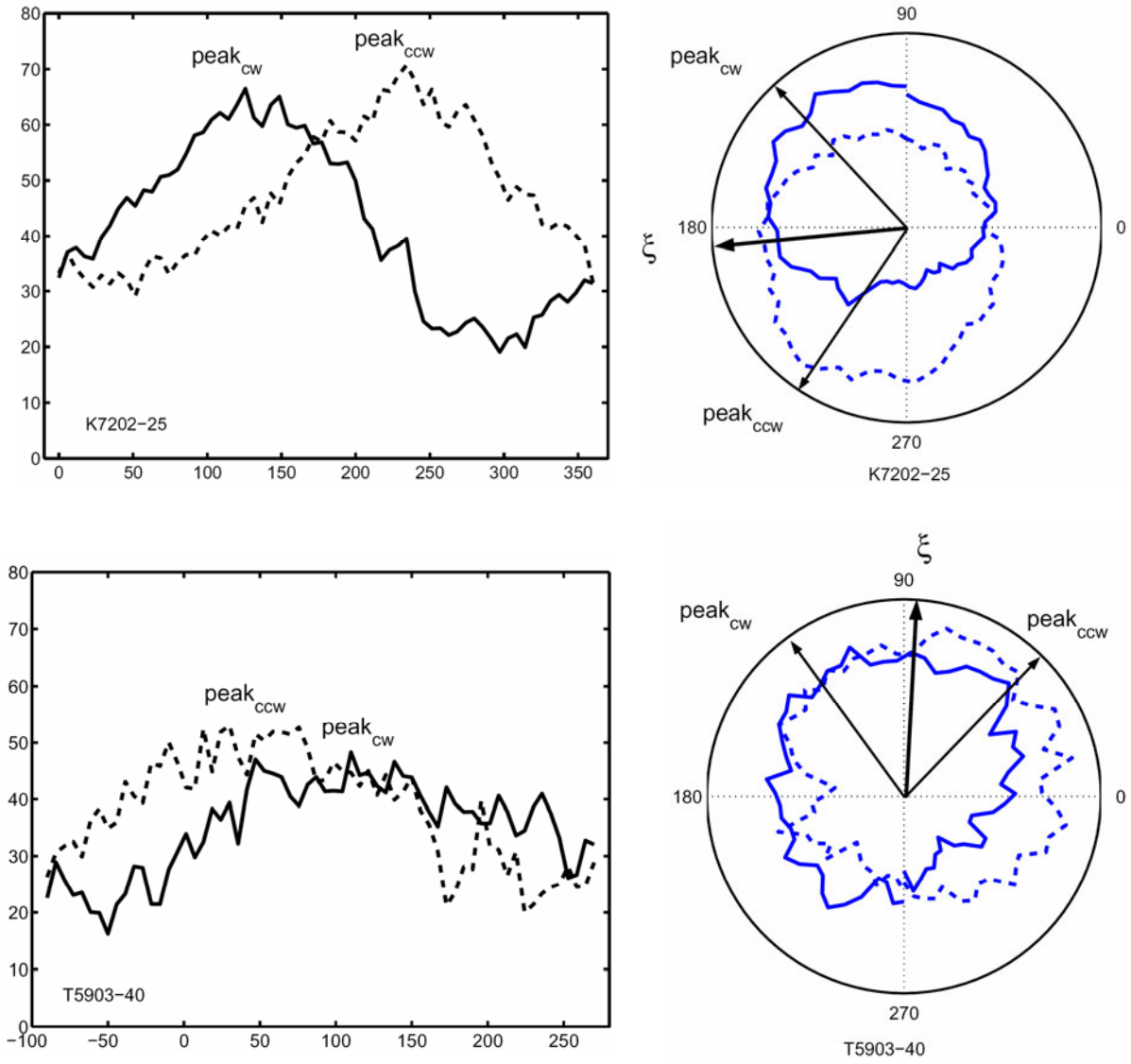

Figure 2.9 Sample unit responses and calculation of orientation and phase parameters

These plots show the unit response to the rotating linear acceleration stimulus due to gravity during CW and CCW rotation, plotted in head-centered coordinates. In the polar plot, left ear-down tilt is to the left; nose-down tilt is upward. Unit K7202 (upper panels) showed an orientation of 185º and a 50º phase lead during OVAR at 25º/s (0.07Hz). The orientation vector of this unit is located near left ear-down tilt. During CW rotation, the stimulus vector sweeps CCW about the head, and the peak response leads the stimulus, occurring between nose-down tilt and left ear-down tilt. During CCW rotation, the stimulus vector sweeps CW about the head, and the response peak occurs between occiput-down tilt and left ear-down tilt, again leading the arrival of the stimulus vector at the orientation vector of the unit at left ear-down tilt. Unit T5903 (lower panels) had an orientation near nose-down tilt, and a 35º phase *lag* during 40º/s OVAR.

Figure 2.10 summarizes the orientations, gains, and phases of one-dimensional unit responses across all OVAR conditions, and mean results for all units. The orientations of these 48 units showed an obvious preference for LED tilt, with 21 units (44%) having orientations within 45º of

left-ear down. Recall that all units were located in the left PB. Fourteen units (14/48; 29%) had orientations within 45º of RED tilt, and six and four units had responses near occiput-down and nose-down tilt, respectively. The majority of units (34) had moderate phase leads. Because gain, phase, and orientation were nearly flat for the majority of units.

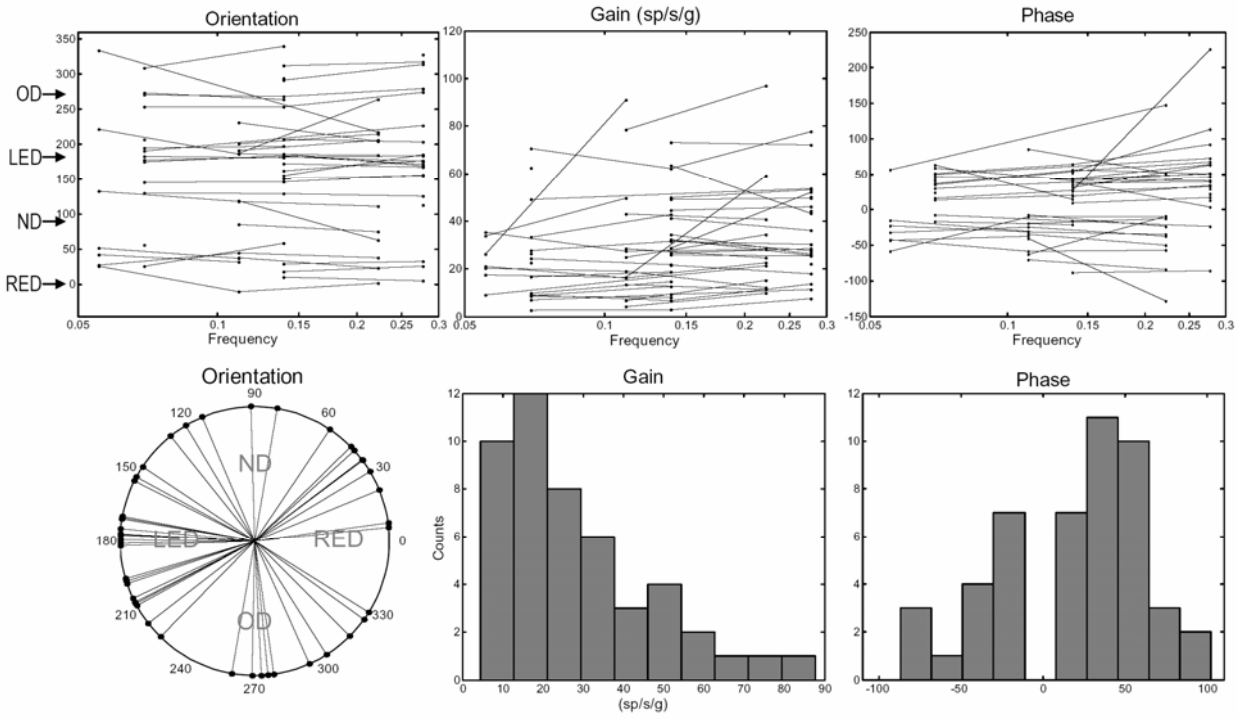

Figure 2.10 Response parameters of 1-D units

Upper panel: Orientation, phase, and gain parameters across all OVAR frequencies tested for 48 linear, one-dimensional units. *Lower panel*: Polar plot of mean orientation parameters, and histograms for average gains and phases for all OVAR frequencies for 48 one-dimensional units. Positive phase values represent phase leads.

2.7.4. Responses to OVAR stimulation. II. Two-dimensional and bias responses:

Two general groups of responses were observed, which did not fit the model of simple linear, one-dimensional responses to OVAR. The first of these response types has been described as being due to linear summation of convergent inputs from motion sensitive inputs with different spatial and temporal characteristics onto units with such "STC" (i.e. Spatio-Temporal-Convergence)-like behavior (Baker et al., 1984; Wilson et al., 1986; Kasper et al., 1988). One

group of units showed significant modulation at the stimulus frequency during one or more OVAR conditions, but significantly reduced or otherwise altered responses during other OVAR conditions. The second group of units showed no modulation under any OVAR conditions, but instead showed a significant change in mean firing rate relative to the direction rotation.

2.7.4.1. Modulated responses:

One type of unit response to bidirectional OVAR showed different modulation amplitudes during oppositely directed rotations. Such responses have been recognized as suggestive of spatio-temporal convergence (Baker et al., 1984; Wilson et al., 1986; Kasper et al., 1988), or convergence of inputs from units with different spatial and temporal response properties, but which are likely to show linear, one-dimensional responses themselves. A second type of response interpreted as inconsistent with the on-dimensional response model showed a significant modulation of firing rate at a frequency other than the stimulus frequency. Examples of both responses are given in **Figure 2.11**.

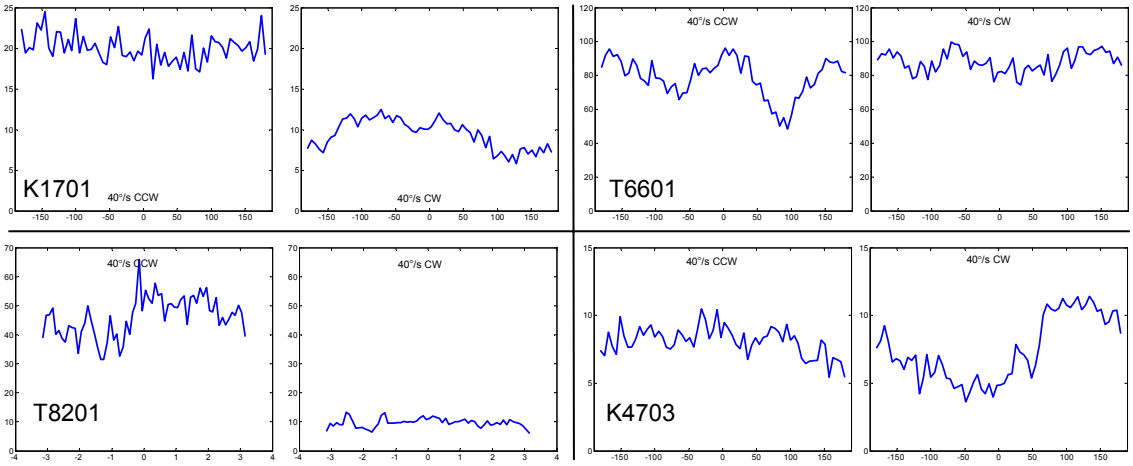

Figure 2.11 STC-like responses with modulation

Responses of four units to OVAR to bidirectional rotation at one frequency. Unit K1701 had an elevated firing rate and no significant modulation during CCW rotation, and a lower firing rate with significant modulation at the stimulus frequency during CW rotation. Unit T8201 had an elevated firing rate and apparent modulation during CCW rotation, and a lower firing rate with no significant modulation during

CW rotation. Unit T6601 had an apparent modulation at twice the stimulus frequency. Unit K4703 showed significant modulation during CW rotation modulation during CCW rotation which was less than half the amplitude of the CW modulation gain, with no significant change in mean firing rate.

2.7.4.2. Bias responses:

One type of unit response to OVAR which has not often been described in the vestibular nuclei (at least not of the magnitude present in our data; however see Reisine & Raphan, 1992) consists of a lack of modulation combined with a significant change in mean firing rate related either to the speed or the direction of rotation. To examine differences in mean firing rate produced by bidirectional OVAR in each class of units (modulated or unmodulated) during each OVAR direction (CW vs. CCW rotation), bias responses for each unit were averaged for responses to CW rotation at all available frequencies, and for responses to CCW rotation at all available frequencies. **Figure 2.12** shows these comparisons for each class of units.

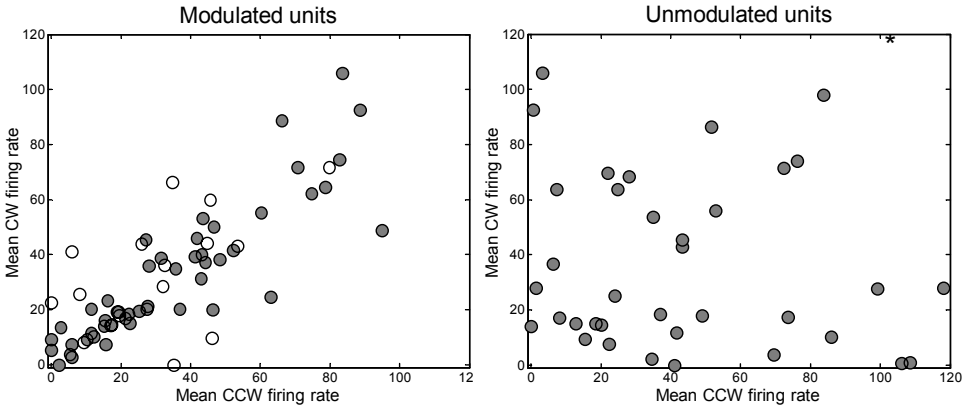

Figure 2.12 Mean firing rate during bidirectional OVAR

Left panel: Mean firing rate during CCW and CW OVAR for all *modulated* units. *Filled symbols*: unit treated as *one-dimensional* units; *open symbols*: units showing OVAR responses suggesting spatio-temporal convergence. *Right panel*: Mean firing rate during CCW and CW OVAR for all *unmodulated* units. One unit (*) with a mean firing rate during CW rotation of approximately 170sp/s and is not shown.

The mean firing rates of *modulated* units showed very good correspondence during bidirectional OVAR (Pearson's r=0.826). However, mean firing rates of unmodulated units were uncorrelated during bidirectional OVAR (r=0.128). Thus, responses of most unmodulated units discriminated between the two directions of OVAR, whereas the direction of rotation was not represented in

the background firing rates of most modulated units. Examples of this type of response are shown in **Figure 2.13**.

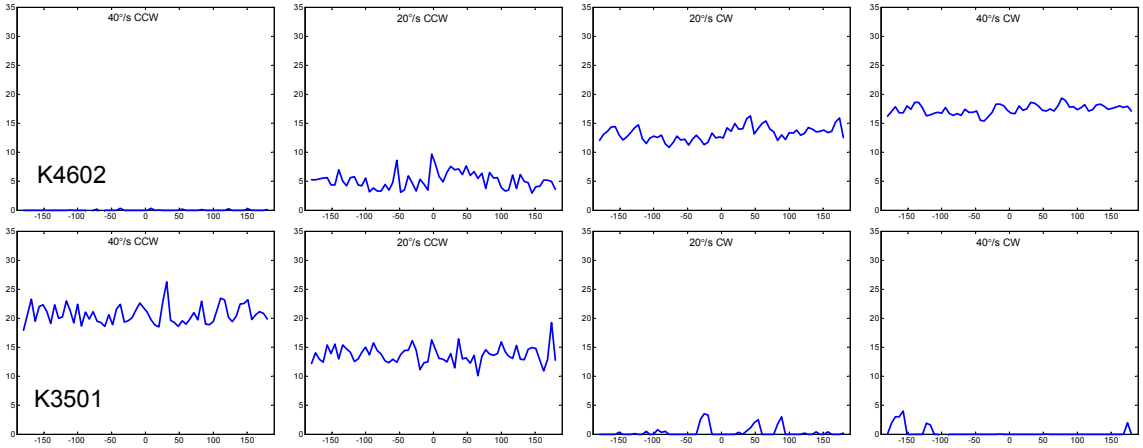

Figure 2.13 Common bias response patterns

Two bias response patterns found during bidirectional OVAR stimulation. *Left columns* show responses to CCW OVAR at two velocities, as labeled. *Right columns* show responses during CW OVAR. *Upper panel*: CCW rotation preferring unit. *Lower panel*: CCW preferring unit. *Solid lines*: estimated baseline firing rate; *dashed lines* mean firing rate during OVAR condition. Responses are plotted over two cycles of rotation. Rotation direction (CW/CCW) refers to direction of table rotation.

For the *unmodulated* units, the influence of the direction of rotation during OVAR was examined further by comparing the mean and standard deviation of firing rates during CW and CCW rotation at the highest velocity available for each unit. Responses were considered to differ significantly from one another if the difference in mean firing rate was greater than two standard deviations. Because separate standard deviations were associated with each mean rate measure, the standard deviation chosen for each comparison was the largest of the two. Among units classified as *unmodulated* (n=38), approximately one quarter (n=11) appeared to be *insensitive* to rotation, with background firing rates across all OVAR conditions within two standard deviations of one another.

A "bias" response, or a significant change in mean firing rate during rotation in one direction versus the other was seen in the remaining units. Approximately three quarters of the recorded units (n=27) had significantly different firing rates during CW and CCW rotation, as determined by comparison of mean firing rates and standard deviations, and were classified as having directionally-selective *bias* responses. Units classified as *directional* were further separated into 'CW' and 'CCW' units (**Figures 2.13**; **2.14**), where CW/CCW refers to the direction of table rotation producing the higher firing rate. *Directional* units with *CW* responses (n=14) showed an increase in mean firing rate during all CW OVAR conditions when compared to all CCW OVAR conditions, while the mean firing rate of *CCW* units (n=13) showed the converse behavior (**Figure 2.14**). Recall that all units were recorded from the left PB, so CCW rotation is toward the side of the recording.

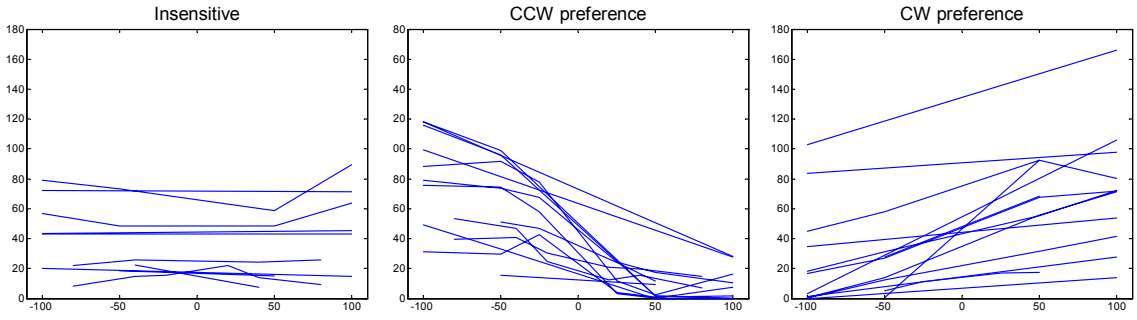

Figure 2.14 Bias responses in unmodulated units during bidirectional OVAR

Mean firing rates of unmodulated units across all tested OVAR conditions. Three classes of units were distinguished based on whether the units showed significant differences (greater than two standard deviations) in firing rate during bidirectional rotation at the highest speed tested. CCW- and CW-preferring units showed a systematic bias in their firing rate in response to the direction of rotation, with an excitatory response to rotation in the indicated direction. *x-axis*: rotation velocity—CCW is to the left, CW to the right. *y-axis*: mean firing rate.

2.7.5. Comparison of unit responses to position trapezoids and OVAR

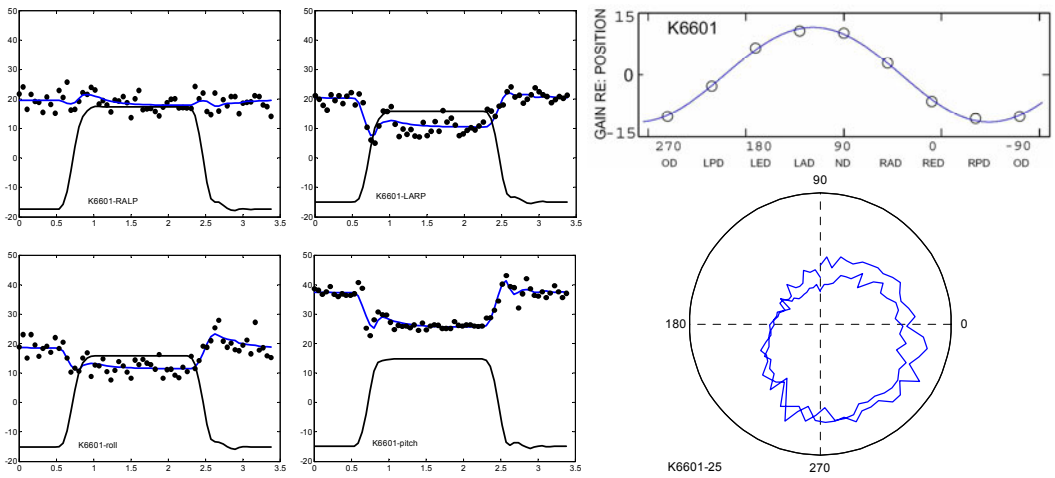

Figure 2.15 Comparison of OVAR and position-trapezoid estimates of response orientation

Responses of one unit to position trapezoid stimulation in pitch, roll, and approximate vertical canal planes (RALP and LARP, as labeled). See text for explanation. *Upper right panel*: plot of position gain parameter used to fit the unit response across all vertical plane trapezoid stimuli. *Lower right panel*: polar plot of firing rate during CW rotation (solid trace) and CCW rotation (broken trace), as presented in Figure 2.9. CW rotation results in rotation of the linear acceleration vector CCW about the head.

Figure 2.15 present the response of a single unit to both position trapezoids and OVAR at on frequency**.** Positive rotations in the plots of position trapezoid responses refer to 'upward' rotations, so that, e.g. positive tilts in the LARP plane are left anterior canal-*up* and right posterior canal-*down*. During roll-plane rotation, the animal was rotated 90º CW with respect to the pitch/roll axis, so that positive rotations in the roll plane result in left ear-up/right ear-down tilt. This unit had a minimal response to stimulus components other than static tilt, but still showed a nearly 180º difference in the response orientation estimated by responses to tilts and OVAR. Such responses were common. The best response to brief tilts resulted from tilts in the direction of the left-anterior canal. Responses to bidirectional OVAR suggested the response orientation was near the right posterior canal –down tilt, and the unit showed a small phase lead. Note that such a response is indistinguishable from a response orientation near left-anterior canal-down tilt with a large phase lag during OVAR.

Linear acceleration responses:

Figure 2.17 shows the orientations of all response components estimated from responses to position trapezoids. Units were classified according to their responses to OVAR. **Figure 2.16** shows a comparison of linear acceleration response gains and response orientations estimated by unit responses to OVAR and position trapezoids for 33 units with one-dimensional responses to OVAR and available static tilt response gain estimates from responses to position trapezoid stimulation. For these units, correlations between response gain estimates were poor (Pearson's r: 0.39), and the orientations of peak responses were actually anticorrelated (Pearson's r: -0.43). The (poor) correspondence between position trapezoid and OVAR linear acceleration response vector estimates is also evident upon examination of the plots of these vectors for one-dimensional units (**Figures 2.10** and **2.17)**, in which they appear to be quite differently distributed. Note that several units with good response gains during OVAR have little or no measured static tilt response during position trapezoid stimulation. A further consideration suggesting the superior reliability of the OVAR stimulus for estimation of linear acceleration response gains is consideration of **Figures 2.8** and **2.10** which both show quite stable response gain estimates from responses to repeated stimulus presentations. It seems unlikely that variability in unit responses to OVAR stimulation is the source of the difference between the two estimates of linear acceleration response gain presented in **Figure 2.16**. Rather it seems much more likely that linear acceleration-like responses to brief static tilts presented by position trapezoids are either extremely variable or perhaps that the responses elicited by these two stimuli are due to sensitivities to different stimulus components.

Angular velocity responses:

One relationship between OVAR responses and angular velocity responses to position trapezoids was evident: The orientations of angular velocity responses were evidently clustered near left-ear down tilt for units with CCW bias responses to OVAR (**Figure 2.17)**, and were clustered near left anterior-canal down tilt for units with CW bias responses to OVAR. No other relationships were evident in comparisons of responses between these two stimuli.

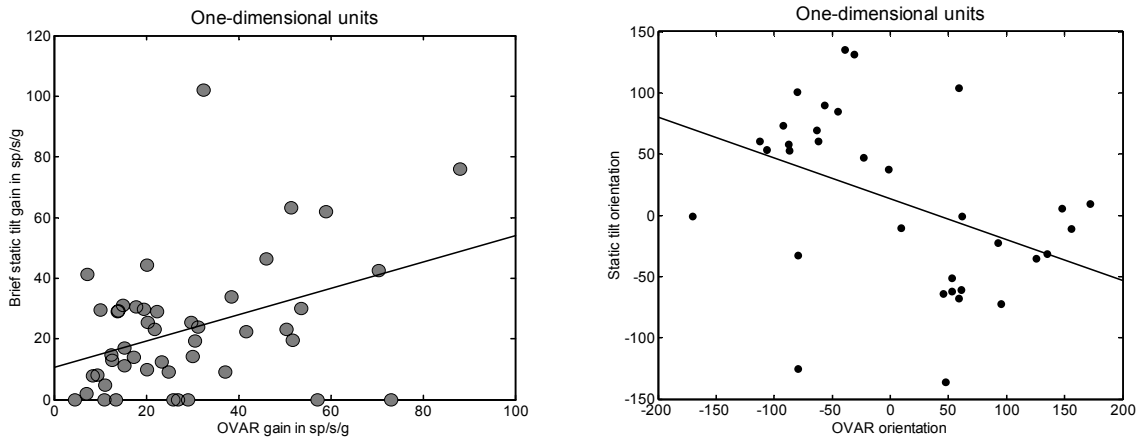

Figure 2.16 Comparison of linear acceleration sensitivity and orientation estimates from OVAR and trapezoids

Left panel: linear acceleration response gain estimates from OVAR and position trapezoids. *Right panel*: estimates of response orientation.

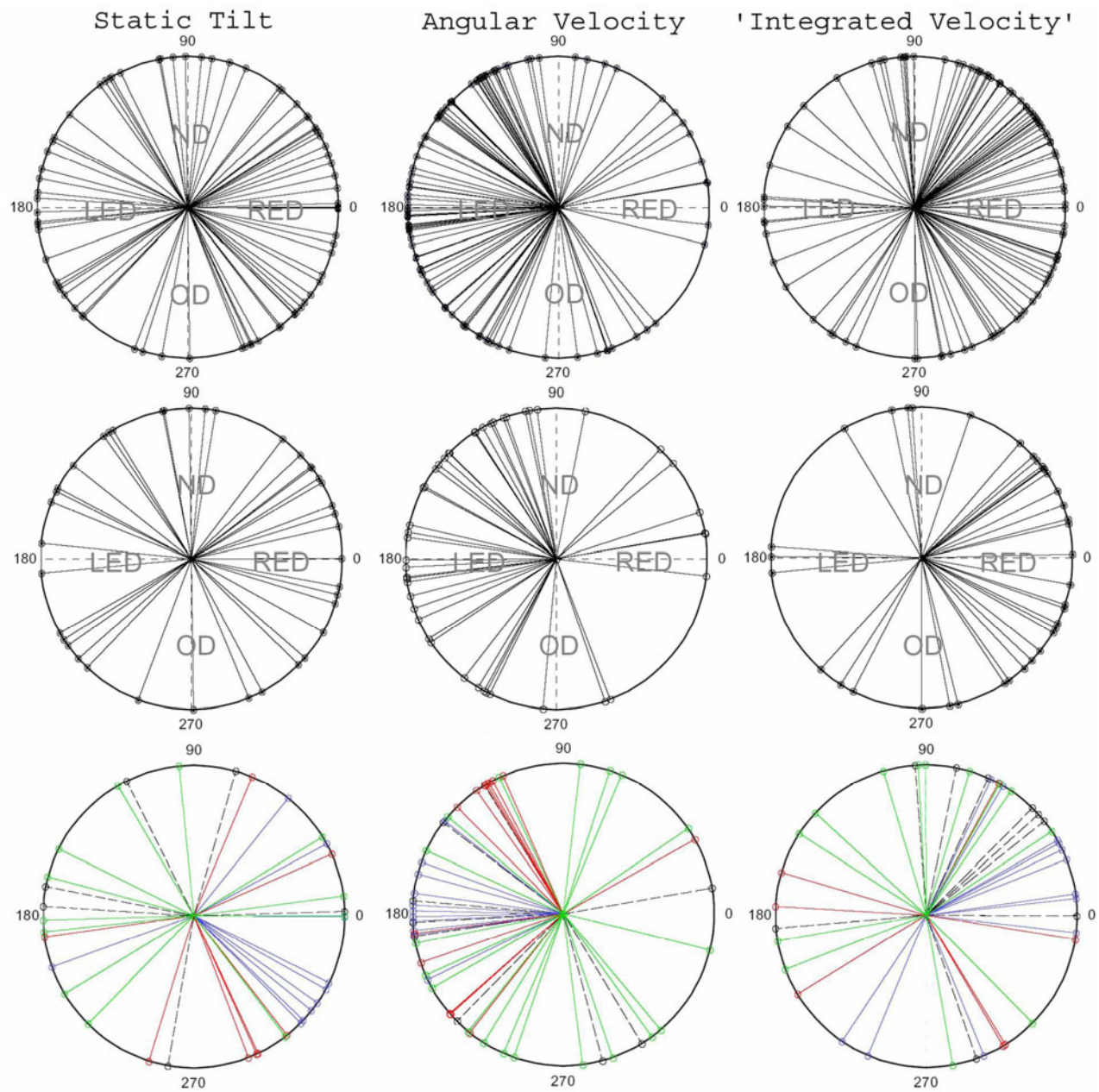

Figure 2.17 Comparison of position trapezoid response component orientations across OVAR response classes

Top row: response component orientations for all recorded units. **Middle row**: units characterized as one-dimensional linear acceleration-sensitive units, on the basis of OVAR responses. **Bottom row**: units characterized as insensitive or showing significant bias responses. *Blue vectors*: units with CCW bias responses during OVAR. *Red*: units with CW bias responses. *Green*: units with other modulated responses to OVAR suggesting STC behavior. *Black dashed lines*: insensitive units. The orientations of static tilt response vectors were approximately uniformly distributed (Rayleigh test: $p>0.45$).

2.7.6. Locations of recorded units:

Units responsive to whole-body motion were located in approximately the lateral two-thirds of the parabrachial complex. All recorded units were located in the posterior two-thirds of PB based on electrophysiological characteristics of neighboring regions. In particular, the anterior limit of the sampled region in all animals was the trochlear nerve root. Animal L was used in the prior study (Balaban et al., 2002), but the six units from animal L reported here were not reported in the prior publication. The six units from animal L which are included here were located among the lateral third and rostral third of the units reported in that study.

Reconstruction of unit locations in animal K was based on electrode tracks found in histological sections, and records of electrode coordinates from recording sessions. Due to a failure of the implant securing the recording chamber to the head, it was not possible to place a marker, such as a lesion, in a precise location in the grid of electrode penetrations prior to sacrificing the animal. Therefore the most medial, lateral, anterior and posterior electrode tracks visible in sections were used as reference points. In addition, the distinctive electrophysiological characteristics of surrounding areas used to locate PB during recordings ensured that all recorded units were within the boundaries of PB. More precise localization was possible in animal L, and the locations of recorded units in that animal were discussed in the prior publication (Balaban et al., 2002).

Animal T is involved in ongoing experiments, so histological reconstructions are not available. However, due to of the placement of the implanted chamber in animal T, only the medial 1mm of PB was accessible at the maximal mediolateral excursion of the x-y stage. The medial edge of PB was easily recognized in multiple penetrations in each animal by 1) a failure to encounter responsive units, 2) a failure to encounter the fibers of the brachium, 3) penetration of the fourth ventricle.

In animals K and T, the fourth ventricle was encountered in penetrations approximately 0.5mm medial to the medial edge of the region in which responsive PB units were found. As a result, the mediolateral coordinates of units in these two animals could be used as a reference.

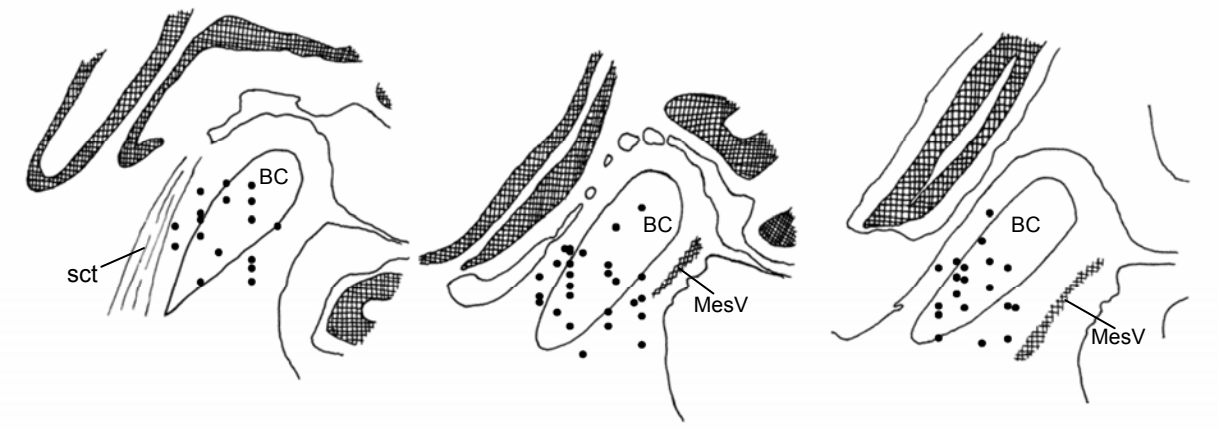

Figure 2.18 Location of recorded units

Approximate location of 68 units recorded from animal K, from caudal (left) to rostral (right). In this figure, the dorsoventral coordinates of units plotted on each section are aligned with the coordinates in the other sections. Sections were cut in a plane tilted approximately 15º posterior from the stereotaxic coronal plane, to approximate the angle of electrode insertion. The centroid of the coordinates of units located in the most caudal section is slightly dorsal to the centroid of unit coordinates in more rostral sections, consistent with the orientation of the PB relative to the angle of electrode tracks. *BC*: brachium conjunctivum—borders of BC are approximate; *sct*: spino-cerebellar tract; *MesV*: mesencephalic trigeminal nucleus.

2.7.7. Topographic distribution of linear acceleration response vectors in PB:

The data presented here were recorded from the posterior two-thirds of the parabrachial complex, caudal to the trochlear nerve root. For the 48 one-dimensional units described above, units showed a tendency to be differentially distributed within this region of the nucleus as a function of their best response orientation (**Figure 2.19**). The dorsoventral and mediolateral coordinates of the units were correlated with the interaural component of the best response vector for each unit. The correlation was significant for the dorsoventral coordinate (Pearson's *r*: 0.342; Bonferroni-adjusted $p<0.05$) but not for the mediolateral coordinate (*r*: 0.282; $p>0.1$). Among

unmodulated units, there were no significant differences in the locations of units with mean firing rate biases showing a preference for CW- or CCW-rotation.

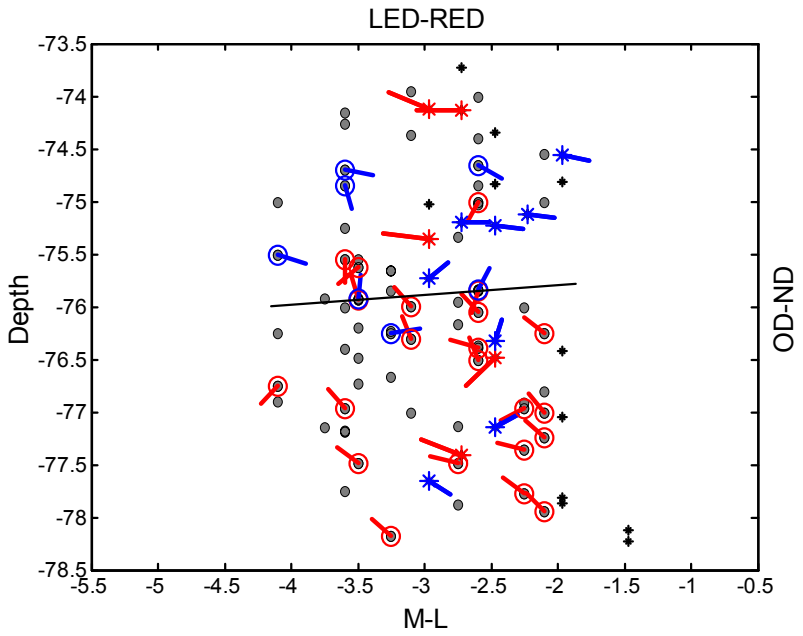

Figure 2.19 Topographic distribution of orientation vectors

Mediolateral and depth coordinates of all recorded units (n=103) in animals K (circles) and T (asterisks). For the 48 units classified as one-dimensional, cells with best response orientations in the ipsilateral-ear down direction (left-ear down) were located more ventrally and tended to be located more medially than units with best response directions in the contralateral-ear down direction. *x-axis*: Mediolateral coordinates of recorded units within the left PB. *y-axis*: depth. Orientation vectors for each unit are plotted with the origin at that unit's coordinates. Units and orientation vectors with any left-ear down component are plotted in red; right-ear down units are plotted in blue. Left-ear down tilt is to the left; nose-down tilt is to the top of the figure. The plotted line shows an approximate border between the two groups of units.

Units from animal T were included in this analysis. Because animal T is involved in ongoing experiments, histological reconstructions were not available for direct comparison. However, the medial edge of the responsive region of PB, as well as the caudal edge of the trochlear nerve, has been defined in both animals T and K in the course of 89 and 148 electrode penetrations respectively, allowing us to compare the relative locations of units within the responsive region of PB in each animal.

In order to compare the relative locations of units with different response properties, the three-dimensional coordinates of recorded units in animal T were adjusted by equating the

medial edge of PB in both animals (see **Section 2.7.6**), and by shifting the dorsoventral coordinates (depth) of units in animal T so that the means of dorsoventral coordinates in both animals were equal. This procedure provides the best possible comparison between animals in the absence of histological data from animal T. One concern must be addressed to support this approach: whether the dimensions of the region in which responsive units were found is approximately equal in both animals. If the size of the sampled regions differ between animals, it is possible that large errors could occur. Because we only wish to support a conclusion about the dorsoventral distribution of units, only this coordinate needs to be considered. In both animals, the dorsoventral dimension of the area in which responsive PB units were identified was approximately equal (**Figure 2.20**).

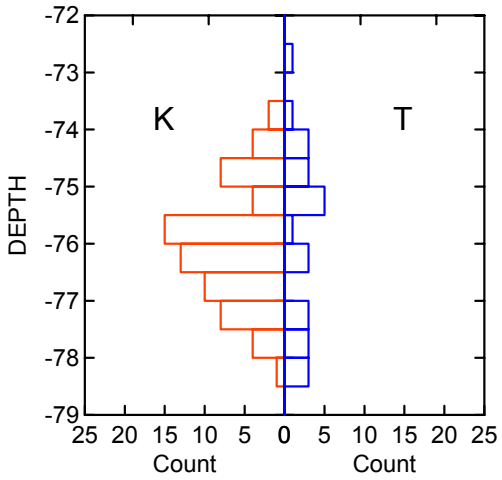

Figure 2.20 Dorsoventral coordinates

Histogram comparing the dorsoventral coordinates of recorded units in animals K and T, showing that the dorsoventral extent of the responsive region of PB was approximately equal in both animals.

2.8. Discussion

2.8.1. Position trapezoid responses

Although it is not possible to draw firm conclusions regarding the sources and nature of responses to position trapezoids, the model proposed by Balaban et al. (2002) was successful in fitting unit responses to the position trapezoid stimulus. This model includes response components to angular velocity, a leaky integration of angular velocity, and linear acceleration (i.e. static tilt). The current study has further investigated unit responses to linear acceleration by examining responses to OVAR. OVAR responses confirmed the general hypothesis that PB units are sensitive to linear accelerations in the head-horizontal plane, as suggested by results from position trapezoid stimulation, but also found significant differences between responses to these stimuli.

Responses to brief static tilts:

Peterson, (1970) found that responses to tilt had both 'phasic' and 'tonic' components. Phasic responses to the initial tilt normally had the same orientation as tonic tilt responses, but were of greater magnitude. Most studies of linear acceleration sensitivity have used longer-duration tilts, rotating linear acceleration vectors, or sustained centrifugal force stimuli, to obviate the difficulties in interpretation of static versus dynamic tilt responses. The present study supports such approaches, as there was extremely limited agreement between linear acceleration response parameter estimates to brief static tilts and OVAR stimuli across all response parameters examined, while for one-dimensional units, there was excellent agreement between bidirectional stimuli and between multiple stimulus presentations over a narrow, two-octave frequency range.

Responses to tilt transients

Responses of units to angular velocity transients were fit by the model proposed by Balaban et al. (2002), which was successful in fitting all unit responses with good results in terms of reduction of variance in the response traces. However, it was not possible to support firm conclusions regarding the origins of transient responses, partially due to the complex nature of the stimulus, and partially due to the significant temporal overlap of the response model components modeled as angular velocity and a leaky integration of angular velocity (see **Chapter 1**). Examination of spatial relationships between these two response components suggested that they may arise from a single source, as the orientations of rotation stimuli resulting in maximal gain coefficients for each model component were approximately opposed. In particular, responses to rotation transients as characterized by angular velocity model components were predominantly in the roll plane, with most units showing angular velocity response vectors near ipsilateral ear-down roll, which is generally consistent with angular velocity inputs from canal-sensitive cells in the vestibular nuclei, where angular responses to ipsilaterally-directed rotations predominate. However, transient response components modeled as being due to a leaky integration of angular velocity tended to respond best to rotations directed opposite to those producing the best angular velocity-like responses, making the determination of response magnitudes difficult to interpret. Further studies of angular velocity responses in isolation, such as by the use of stimuli consisting of prolonged angular accelerations and periods of constant angular velocity (Goldberg & Fernandez, 1971) or in the context of a more tractable stimulus and response model are necessary to determine the sources and nature of these two response components before it will be possible to confidently estimate the magnitude of response components for which they account.

2.8.2. OVAR responses

Numerous human and animal studies using OVAR have demonstrated that perstimulus eye velocity can be decomposed into bias and modulation components (e.g., Guedry 1965, 1974; Darlot et al. 1988), and responses of units in the rostral pole of the vestibular nuclear complex have been described similarly and correlated with eye-movements (e.g., Reisine and Raphan 1992). The bias component is invariant across head orientations during OVAR, while the modulation component is a perstimulus cosinusoidal variation in eye velocity or unit discharges with changes in head orientation. We have demonstrated that the discharges of neurons in the vestibulorecipient portion of the primate parabrachial nucleus during constant velocity OVAR also display bias and modulation response components. These bias and modulation signal components appear, to a first approximation, to be distributed independently among PB units. Similar results were reported in macaque vestibular nuclei: different horizontal and vertical semicircular canal-responsive vestibular nucleus units showed either bias-only or modulation-plus-bias response components during OVAR stimulation (Reisine and Raphan 1992). Hence, the existence of independent bias and modulation components in OVAR responses of units in the vestibulo-recipient region of the parabrachial nucleus is consistent with bilateral projections from the vestibular nuclei (Balaban et al. 2002; Balaban 1996, 2004; Porter and Balaban 1997).

Unit responses to OVAR appear to represent different combinations of bias and modulation components. Sixty-five units in this sample showed a perstimulus modulation in firing rate during OVAR, with mean firing rates which were well-correlated during bidirectional rotations. An increased bias response component during rotation may be expected to increase the dynamic range for modulation responses. However, for all units, background firing rates and modulation response gains were poorly correlated. Across all recorded responses to OVAR

(n=406), mean firing rates and modulation gains were uncorrelated (Pearson's product-moment correlation: 0.03).

The bias responses of the majority of units in both experiments (76/103; 74%) were not directionally selective, showing similar mean firing rates during CW and CCW rotation at all velocities tested. Twenty-seven remaining units had directionally-selective bias responses. This finding differs from the lateralized, eye-movement related behavior of vestibular nucleus units in macaques (Reisine and Raphan 1992), which were reported to increase their firing rates during ipsilateral rotation and decrease firing during contralaterally directed OVAR. The bias responses of parabrachial nucleus units are a linear acceleration-based signal of constant rotation speed that persists long after adaptation of the semicircular canal response. No systematic relationship between bias and modulation response magnitudes was found, although it is possible that bias responses increase the dynamic range of some PB neurons to accommodate increases in modulation response gains during rotation (e.g. unit T8201 in **Figure 2.11**). However, units with asymmetric bias responses are clearly capable of indicating the direction of continuous OVAR following adaptation of the horizontal canal angular velocity response, and it is possible that PB units with asymmetric OVAR modulation amplitudes contribute to the discrimination of direction, as has been suggested for otolith-sensitive vestibular nucleus units in the cat (Chan 1997; Lai and Chan 1995).

Of the neurons in this sample which did not show a significant perstimulus modulation in firing rate during OVAR, 27/37 units showed bias-type responses, with significant changes in mean firing rate related to the direction of rotation (ten for CW rotation, seventeen for CCW rotation). Thus, activity patterns in the PB appear to reflect both the direction of tilt (head-

referenced horizontal linear acceleration) and the direction and rate of rotation (yaw angular velocity).

2.9. Potential sources of OVAR modulation responses:

Schor et al. (Schor et al. 1984) recorded from vestibular units in cats in which all semicircular canals had been plugged (thus removing any influences of semicircular canal-derived angular velocity inputs to the recorded units), and demonstrated that the linear acceleration response vectors of vestibular nucleus neurons as assessed by sinusoidal tilt in several vertical planes correspond closely to the orientation vector determined with bidirectional constant velocity 'wobble' stimulation, which is physically equivalent to OVAR in terms of the imposed rotating linear acceleration vector due to gravity. However, OVAR stimulation obviates the need for dafferentation of the canals, as it imposes no rocking motion in the pitch and roll planes. Therefore, subsequent studies of vestibular nucleus units (Lai and Chan 1995; Chan 1997; Chan et al. 2002), reticular formation units (Chan et al. 1996) and cells of the cerebellar anterior vermis (Manzoni et al. 1995) in decerebrate animals have used OVAR to characterized the spatial and temporal properties of unit responses to linear accelerations using periodic stimulation of the otolith organs in the absence of canal stimulation.

It is known that the majority (~75%) of horizontal linear acceleration responses in the VIIIth nerve and in the vestibular nuclei are excitatory to ipsilateral ear-down tilts and inhibitory to contralateral ear down tilts, and that this pattern of responses is reinforced by commissural inputs from the contralateral labyrinth (Fujita et al., 1968). In the current study, 21 of the 48 'one-dimensional' units had orientation vectors near ipsilateral ear-down tilt, and 14 units had orientations near contralateral ear-down tilt, consistent with the existence of bilateral vestibulo-parabrachial projections. The current study analyzed unit responses to OVAR as one-

dimensional linear accelerometers, and found responses consistent with those seen in other central vestibular neurons. We conclude that the presence of such responses in PB is consistent with an origin of PB linear acceleration sensitivity responses in the utricle.

2.10. Bias responses to OVAR stimulation:

Persistent changes in background firing rates ('bias') and asymmetric modulation responses during bidirectional OVAR have been interpreted as being consistent with convergent inputs from motion sensitive units with different spatial and temporal responses properties and have been observed in vestibular nucleus units (Baker et al., 1984; Kasper et al, 1988; Angelaki, 1992a,b,c; Reisine & Raphan, 1992; Lai & Chan, 1995), and termed "spatio-temporal convergence" (STC). The present study suggests the presence of STC type responses in PB, consistent with convergence of afferents from the vestibular nuclei on single cells in PB. The modulation responses are rather more straightforward and have been encountered in a number of studies.

Bias-type responses, however, are less commonly encountered (Reisine & Raphan, 1992), and it is tempting to assume that they play a role in the generation of biases in slow-phase eye velocity during persistent nystagmus produced by OVAR, as this is a prominent feature of eye-movement responses to OVAR (Guedry, 1965; Darlot et al., 1988) without a ready explanation in the firing patterns of most central vestibular units or in a straightforward model accounting for sensitivity to continuous rotation (we accept that the data presented by Reisine & Raphan did show excellent evidence of eye-movement related responses, but we do not have the necessary data to properly answer this question—please see **Section 3.3.1** for a more detailed discussion of this subject). As a result, rather obtuse approaches have been taken to the explanation of eye-velocity bias generation on the basis of known linear-acceleration sensitive

central vestibular responses whose relationships to constant angular velocity estimation from the linear acceleration sensitivity of the utricle are not immediately obvious (Raphan & Schnabolk, 1988; Angelaki, 1992c), including postulations of sensitivity to torsion forces acting on the otoconia, and responses to stimuli orthogonal the plane of the utricle. We will not attempt to interpret these stuidies here however, because, as was discussed in **Chapter 3**, we are unable to draw conclusions regarding the role of PB units in the generation of eye-velocity bias and modulation during OVAR due to a lack of data regarding either unit firing during VOR-cancellation or during optokinetic stimulation. However, prior study of smooth pursuit eye movements (C.D. Balaban, personal communication), and routine observation of unit firing and eye movements during both exploration and mapping of the borders of PB (which is bordered by areas with prominent eye-movement responses such as the trochlear nerve and abducens nucleus), and recordings within PB demonstrated that, at the least, parabrachial complex unit responses are unrelated to the quick phases of nystagmus or to pursuit and saccadic eye movements. Future studies may employ either the VOR-cancellation paradigm or optokinetic stimulation to examine relationships of unit firing to eye-movements more closely. Indeed, inspection of the histological reconstructions provided by Reisine and Raphan (Reisine & Raphan, 1992), suggests that they may have unwittingly recorded responses of units in the parabrachial nucleus, as their electrode tracks appear to pass through this structure.

2.11. Conclusion:

A model has been elaborated by Balaban and Thayer (Balaban & Thayer, 2001; Balaban, 2002), which proposes that neurons of the PB (parabrachial complex, including the parabrachial and Kölliker-Fuse nuclei) are a key component of a network that connects the vestibular nuclei (VN) with brainstem and forebrain areas involved in autonomic, nociceptive and affective processing,

particularly the extended amygdala and insular, infralimbic, and lateral frontal cortex, as well as the thalamus and hypothalamus. This network was suggested as a neural substrate for clinically-observed linkages between balance disorders and disorders of affect, as well as normal affective responses to challenges to spatial orientation (Balaban & Jacob, 2001). The responses of PB units to linear acceleration stimuli produced by OVAR and the correspondence between these responses and responses of known linear acceleration-sensitive central vestibular neurons supports the hypothesis that PB is a functional participant in brainstem vestibular networks. Further work in PB should allow the establishment of specific physiological relationships between brainstem vestibular mechanisms and the known targets of ascending and descending PB efferents, and the sensory, autonomic and other networks in which it participates.

3. Discussion

As stated in the introduction, the chief objective of this study was to extend the characterization of PB units to whole-body motion by studying linear-acceleration responses using OVAR. The experimental data presented in Chapter 2 supported the following conclusions:

1) That the previous study (Balaban et al., 2002) was correct in its determination that units in the parabrachial complex are sensitive to linear accelerations in the head horizontal plane, consistent with inputs from the vestibular nuclei. Linear acceleration-like responses to brief static tilts noted in an earlier study (Balaban et al., 2002) were correct in terms of the proportion of units reported to respond to linear accelerations imposed by brief whole-body tilts with respect to the gravity vector. In both the previous and current studies, the proportion of units responding to the static tilt component of position trapezoid stimuli were similar. About 60% of the units recorded (Balaban et al., 2002) had a response component which appeared to be due to static tilt. The current study found that 75% of recorded units had significant responses to static tilt. That is, the inclusion of a static tilt parameter in the response model presented in **Chapter 2** (**Methods**) resulted in significant improvement in the variance accounted for by model fits according to the nested-F test, with α=0.01. When we tested for the presence of significant sensitivity to a rotating linear acceleration stimulus using OVAR, we found that a similar proportion of cells (65%) showed modulation at the frequency of the OVAR stimulus under at least one OVAR condition, the magnitude of which significantly greater than the total remaining variance in the unit's firing rate. This proportion was reduced somewhat by the inclusion of more stringent criteria, i.e. rejection of response amplitudes (gain coefficient of the cosinusoidal response model) of <3sp/s and/or VAF<0.4 (**Section 2.7.3**).

However, we also found two sources of disagreement between the linear acceleration-like responses produced by each stimulus. First, the orientations of unit vectors describing the direction of tilt producing the best response differed in their distributions in the head-horizontal plane. In particular, nearly half (21/48) of the recorded units showing responses to OVAR which could be characterized as responses of linear, one-dimensional accelerometers had response orientation vectors within 45º of left ear-down tilt (**Figure 2.10**), consistent with known responses of both primary vestibular afferents and central vestibular neurons. While this aggregation was not strong enough to be considered significantly different from a uniform distribution (Rayleigh test: $p=0.072$), linear acceleration response vectors determined from brief static tilts in these units, showed no such trend whatsoever and were distributed nearly uniformly (**Figure 2.17**; Rayleigh test: $p>0.45$). Second, eight units (17%) showing OVAR responses characterized as one-dimensional failed to show significant static tilt responses during position trapezoid tilts. As discussed in the Introduction, this was likely due to interference between the static tilt and integrated angular velocity components of the position trapezoid response model proposed by Balaban et al. (2002).

2) That the distributions of linear acceleration response orientations assessed with OVAR were consistent with inputs from the vestibular nuclei and primary labyrinthine afferents, where the majority of utricle-sensitive neurons respond best to ipsilateral ear-down tilt (see **Figure 2.10**), with the next largest proportion of units responding to linear acceleration stimuli near contralateral ear-down tilt, and fewer units responding to tilts near the pitch plane (Adrian, 1943; Fujita et al., 1968; Curthoys & Markham, 1971; Peterson, 1967, 1970; Curthoys & Markham, 1971; Loe et al., 1973; Fernandez & Goldberg, 1976; Goldberg et al., 1990).

3) That other responses encountered in the parabrachial complex are consistent with responses of vestibular nuclear units hypothesized to result from the convergence of inputs either from one or more labyrinthine organs, with different spatial and temporal response properties, i.e. spatio-temporal convergence. In addition, we found a significant population of units with prominent 'bias' responses, consistent with behavior of neurons in the superior and medial vestibular nuclei described by Reisine & Raphan, (1992), both of which regions are known to give rise to efferent projections to the parabrachial complex (Balaban, 1996, 2004).

3.1. Responses to tilt transients:

Balaban et al. (2002) recorded from units in the parabrachial complex, and reported that unit responses to position trapezoid stimulation appear to contain response components related to transient velocity or acceleration inputs resulting from brief angular rotations. Interestingly, no units in the previous report responded only to yaw-axis rotation transients (Balaban et al., 2002), while all units had some sensitivity to rotation transients in vertical planes. The findings of the present study agree with this conclusion generally: all but one of the units examined here had some nonzero angular velocity response in vertical rotation planes. In units receiving semicircular canal inputs, we would expect that some proportion of units would receive horizontal canal input in the absence of vertical canal inputs. Such a finding would strengthen the conclusion that the dynamic responses to position transients seen in these studies was due to angular-, rather than to dynamic linear-acceleration sensitive inputs. Units responsive only to yaw-axis angular velocity transients were not found by Balaban et al. (2002), or in the present study, calling into question the angular velocity results generally. Therefore it is necessary to investigate the angular velocity and static tilt responses of PB units separately.

However, the present study examined linear acceleration responses in the absence of angular acceleration, and provided data which differed with results from position trapezoids in the same units, but which agreed with findings regarding linear acceleration responses in other otolith-sensitive central vestibular neurons. Given the noted disagreement between estimates of static tilt response gains and orientations determined by brief trapezoidal tilts and OVAR, and the difficulty in disambiguating the relative contributions of angular velocity-like and leaky integrated velocity-like response components included in the trapezoid response model, we conclude that additional studies aimed specifically at the evaluation of angular velocity and angular acceleration responses are necessary.

As responses to OVAR stimulation provided support for the hypothesis that parabrachial neurons are sensitive to linear accelerations in a manner consistent with input from linear acceleration-sensitive vestibular nuclear units, we support the general hypothesis of Balaban et al. (2002) that some part of the angular velocity-like responses to rotation transients can be correctly attributed to inputs from semicircular canal-sensitive central vestibular neurons. However, the source(s) of velocity and leaky integrated velocity-like response components which clearly contribute to significant improvements in model fits to unit responses cannot yet be attributed uniquely to sensitivities to angular velocity, angular acceleration, or dynamic linear acceleration on the basis of position trapezoid responses.

For example, Fernandez & Goldberg (1976a,c) found that otolith afferents showed responses to dynamic changes in the imposed linear acceleration stimulus which were generally greater than, but of the same sign as responses to long-duration static forces. Transient responses which are similar in character have been reported in studies of responses to brief tilts (Peterson, 1970), and in comparisons of otolith afferent responses to slow, “barbeque spit” rotations (i.e.

rotations about earth-horizontal axes to provide gravity-derived linear acceleration inputs to the otolith organs) versus stepwise rotations about the same axis (Lowenstein & Roberts, 1950; Loe et al., 1973). That is, dynamic stimulation of the otolith organs can result in transient responses to vertical plane rotations which are similar in orientation and sign to static tilt or sustained linear acceleration responses, and responses of this type might account for the dynamic tilt responses seen during position trapezoid stimulation.

3.2. Distribution of response vectors within PB.

A topographic distribution of response vectors has not been described previously in central vestibular areas. This study found a statistically significant difference in the dorsoventral locations of units with linear acceleration response vectors oriented toward contralateral ear-down tilts (dorsal) and ipsilateral ear-down tilts (ventral). Although there is significant overlap in the distribution of these two response types, this finding is quite surprising. It is possible that the differential distribution of response vectors is analogous to other partial topographies the differential localization of taste, nociceptive and other sensory representations within PB, which appear to be related to their differential functional relationships with midbrain and forebrain systems involved in separate e.g. sensory and hedonic or cognitive functions, such as the roughly segregated populations of units with static, (or passive), and plastic representations of taste stimuli which enable the participation of PB in the formation of conditioned taste aversion, as discussed below. Because our knowledge of the anatomical and functional organization of the parabrachial region with respect to the vestibular system is currently very crude, it is impossible to make firm statements regarding the significance of the observed topographical distribution of responses.

3.3. PBN Outputs:

Due to the prominent projections from the caudal aspect of PB to the central and extended amygdala and to thalamic nuclei (Saper and Loewy 1980; Fulweiler and Saper 1984; Krout and Loewy 2000), the contribution of both angular velocity and linear acceleration sensitivities to unit responses could potentially be important for higher-level cognitive and integrative functions (e.g., distinguishing tilt from translation or influencing subjective sensations of spatial orientation and postural stability in the gravito-inertial reference frame during complex movements). Because the lateral parabrachial nucleus is a major component of ascending interoceptive pathways (Craig 2002), it was suggested that these neurons form an integral link in the neural network connecting balance, subjective sensation, emotional expression and affect.

Although it is impossible to know at this stage precisely how PB unit responses to self-motion might relate to activity in amygdalar circuits, according to the scheme of the parabrachial's roles in taste and pain sensation developed in the introduction and below, one can easily imagine that PB unit responses to self-motion participate in a dynamic, plastic circuit involved in learning phenomena and subject to descending influences from forebrain structures such as the amygdala.

3.3.1. Possible relationships with eye movements:

Eye movements were recorded in animals L and K. Search coils for eye movement recording were unavailable during recordings from animal T. In the first report on whole-body motion responses in PB units, Balaban and coworkers (Balaban et al., 2002) reported that one animal had been trained to visually pursue a visual target, and showed no unit activity related to pursuit eye movements. In addition, no recorded units showed any relation to spontaneous saccadic eye movements or eye position, or to quick phases of nystagmus during OVAR.

In the present study, spontaneous eye movements were observed on-line during recordings in animals L and K, and were visually observed during unit identification in animal T, and were again seen to be unrelated to unit firing in any of the recorded units.

However, two possible relationships between unit firing and eye movements may be considered in future studies: responses related to the vestibulo-ocular reflex (VOR), and the bias and modulation components of slow-phase eye velocity during nystagmus produced by OVAR. In order to draw strong conclusions regarding relationships of unit firing to these two types of eye-movements, we would need to unit study responses under two additional conditions: cancellation of the VOR, and optokinetic stimulation. These are discussed below in sequence.

Vestibulo-ocular reflex:

During brief whole-body rotations, the VOR serves to stabilize the eye in the orbit, and thereby stabilizes the direction of gaze and minimizes retinal slip. In order to determine the relationship between unit activity and VOR-related eye movements, it is necessary to use the "VOR-cancellation" paradigm (Lanman et al, 1978; Chubb et al., 1984; Cullen et al., 1991; Cullen & McCrea, 1993; Cullen et al., 1993; Belton & McCrea, 2000). These studies generally examined eye-movement responses of VN units during periodic yaw- or vertical-axis rotations, and trained animals to fixate on a target which moved with the animal's head, thus forcing the animal to cancel the reflex. These studies showed that unit responses during sinusoidal head motions could be systematically related to eye movements, rather than passive responses to semicircular canal inputs due to rotation. In the current study, eye-movements related to the VOR are confounded with angular velocity responses to position trapezoid motion transients. In order to show that unit responses to rotation are independent of reflexive eye movements, it will be necessary to study unit responses under the VOR-cancellation paradigm.

Vestibular and optokinetic stimulation-induced nystagmus:

During OVAR, there exists a maintained per-rotational nystagmus (Guedry, 1965; Cohen et al., 1983). The slow-phase of eye velocity during this nystagmus is modulated at the frequency of rotation, as well as showing an overall bias in slow-phase eye velocity. Reisine and Raphan (Reisine & Raphan, 1992) recorded from neurons in the superior and medial vestibular nuclei and reported that units in this region showed both bias responses (in 'vestibular-only' units which were insensitive to saccadic eye-movements) that followed the timecourse of slow phase eye-velocity bias during OVAR, and modulation responses (in 'vestibular-plus-saccade' units which were sensitive to both angular velocity and saccadic eye-movements) which were of the same frequency as the modulation in slow-phase eye velocity.

While some degree of correlation between the bias and modulation components of slow-phase eye velocity and the bias and modulation components of PB unit responses to OVAR certainly exists in our own data, these findings would not be conclusive as to relationships between unit firing rates and recorded eye movements, for two reasons. 1) In a prior study of superior and medial vestibular nuclear responses to OVAR, the peaks of modulation responses relative to head position varied from unit to unit and were unrelated to the peaks of slow-phase eye velocity modulation responses to OVAR, so these responses could not be conclusively related to one-another (Reisine & Raphan, 1992). In our own dataset, the phases of modulation responses relative to head position during rotation are likewise distributed throughout the head-horizontal plane, and therefore unrelated to the phase of slow-phase eye velocity modulation during rotation. 2) Second, and most importantly, in Reisine and Raphan's study, units showing bias responses to OVAR in the absence of modulation *were not* responsive to saccadic eye movements, but *were* correlated to changes in the bias component of slow-phase eye velocity during optokinetic nystagmus. While all units reported in the present study were unresponsive to

saccadic eye movements, we have not used optokinetic stimulation, so we are unable to draw firm conclusions regarding relationships with slow-phase eye velocity. A specific comparison of eye movement responses to OVAR and optokinetic stimulation will be necessary to resolve this question.

3.4. Diversity of PB responses to whole-body rotations

Teleologically, it is possible that an important feature of the projection from PB to midbrain and forebrain regions involves responses 'tuned' to vestibular stimuli which might elicit strong attentional or affective responses to self-motion not only in disordered individuals, but also in normal subjects presented with challenges to spatial orientation or postural stability. PB neurons are both a potential source of ascending unconditioned vestibular stimulus information to forebrain associative mechanisms and a potential substrate for plastic mechanisms necessary for learning behaviorally-relevant associations between vestibular stimulus configurations and their consequences. The selection and organization of appropriate motor responses to challenging stimuli must be very rapid in order to be successful. Such events would favor an arousal and associative learning system that was immediately informed about the occurrence of potentially harmful constellations of self-motion inputs. Finally, such inputs should be capable of compressing and transmitting information containing sufficient detail to allow for immediate selection between potential behavioral or other organismal responses. The diversity of observed responses of PB units in this and prior work suggest that neurons in PB are capable of providing rich information about stimuli impinging simultaneously upon the semicircular canals and otolith organs. The anatomical position of PB suggests that it is able to relay this information directly to forebrain regions known to be important in attending to threatening stimuli and selecting and organizing responses to such threats, and in the acquisition of learned responses to stimuli

predictive of threats. The hypothesis that the PB acts to relay behaviorally relevant vestibular information to forebrain learning and affective circuits provokes the question of what characteristics of PB unit responses might be included in the forward projections of PB. Further studies using e.g. antidromic identification of parabrachial inputs to CeA are necessary to address this question.

3.4.1. PB Functions supporting a role in vestibulo-affective and –autonomic linkages

A model has been elaborated by Balaban and Thayer (Balaban & Thayer, 2001; Balaban, 2002), which proposes that neurons of the PB (parabrachial complex, including the parabrachial and Kölliker-Fuse nuclei) are a key component of a network that connects the vestibular nuclei (VN) with brainstem and forebrain areas involved in autonomic, nociceptive and affective processing, particularly the extended amygdala and insular, infralimbic, and lateral frontal cortex, as well as the thalamus and hypothalamus. This network was suggested as a neural substrate for clinically-observed linkages between balance disorders and disorders of affect, as well as normal affective responses to challenges to spatial orientation (Balaban & Jacob, 2001).

Neurons in this region appear to form a reciprocal link with the central amygdaloid nucleus (CeA) (Price & Amaral, 1981; Fulwiler & Saper, 1984; Pritchard et al., 2000), with CeA efferents terminating primarily within lPBN and most terminals containing GABA (Jia et al., 2005).

PB receives projections from ipsilateral cerebellar vermis lobule IX, which gives rise to cerebellar corticovestibular projections (Haines, 1975), suggesting convergence of self-motion-related inputs to PB from multiple central vestibular sites, PB projects, with the locus coeruleus, to the central mesencephalic reticular formation (cMRF), which is involved in the control of saccadic eye movements (Sakai et. al., 1977), and to the Edinger-Westphal nucleus, which is

involved in pupillary accommodation (Pick, 1970; Sillito & Zbrozyna, 1970; Loewy & Saper, 1978).

It is possible that PB projections to the vestibular nuclei participate in the influence of affective state upon vestibular sensation, postural control, and vestibular reflex circuits by providing feedback input to the vestibular nuclei to influence the processing of self-motion information, possibly in concert with raphe-vestibular and coeruleo-vestibular projections (Balaban & Thayer, 2001; Balaban, 2002). It is also possible that PB projections to VN play a role in the central processing of self-motion information which is independent from affective processing. These functions are suggested by the well-known relationships of PB with cognitive and affective aspects of sensation and autonomic control in other functional systems.

3.4.2. Known functions of ascending pathways through PB

Several major themes of ascending parabrachial pathway functions are well-established, including nociception, interoception, respiration, gustatory sensation and conditioned avoidance learning. It will be noted in the discussion that follows that a unifying characteristic of several parabrachial functions is that of peculiarly behaviorally-relevant information processing, whether purely sensory in nature or as the subject of some learning process. These observations suggest a role for the PBN in sensory and learning processes related to certain aversive or otherwise motivationally relevant stimuli. In addition, descending parabrachial outputs have been identified as participants in autonomic and visceromotor control, which positions PBN within both input and output streams related to organismal challenges encountered in the environment and the homeostatic responses to them. For reference, the basic scheme of efferent connections in the rat and monkey are presented in Table 3.1.

3.4.2.1. PBN as a pain relay nucleus

The parabrachial nucleus has been considered as a relay nucleus for spino-parabrachio-thalamic, spino-parabrachio-hypothalamic and spino-parabrachio-amygdalar pain pathways (Ma and Peschanski 1988; Bester et al. 1995; Jasmin et al. 1997; Li and Li 2000; Bourgeais et al. 2001). The ventrolateral parts of PBN and the Kolliker-Fuse nucleus, which contain many of the vestibuloresponsive units identified by Balaban et al. (2002) provides a disynaptic link between the dorsal horn of the spinal cord and trigeminal subnucleus caudalis, and the amygdala in rats (Ma & Peschanski, 1988, Jasmin et al., 1997), and is considered a major relay in spinal and trigeminal nociceptive afferent pathways (Cechetto et al. 1985), also terminating in nociceptive regions of the hypothalamus (Bester et al., 1995) and thalamus (Nomura et al., 1978; Ma and Peschanski, 1988). PBN neurons are responsive to noxious cutaneous and visceral stimuli in the noxious range (Bernard et al., 1994), and Craig (1992) has shown that, in both the cat and monkey, lamina I neurons terminate throughout the PBN and K-F, although they appear to be most concentrated within the ventral two-thirds of lPBN and the dorsal/lateral part of mPBN. However, Ma and Peschanski were initially unable to confirm that the lateral parabrachial nucleus, which was known to project to nociceptive thalamic regions, provides a direct disynaptic nociceptive input to thalamic nuclei, prompting them to speculate that the direct spino(trigemino)-parabrachio-amygdaloid nociceptive pathway was more closely involved in the emotional aspects of pain sensation.

The PBN is also important in both the stimulus and analgesic properties of morphine, and non-opioid analgesia has also been shown to be mediated by the PBN. In a study intended to examine the effects of LC lesions on morphine analgesia, Hammond and Proudfit (1980) instead found that only those lesions which included the ventromedial PBN resulted in attenuation of morphine's analgesic effects. The PBN shows dense mu-opioid receptor binding (Atweh &

Kuhar, 1977; Wamsley et al., 1982) and is one of the brain areas most affected by Naltrexone-induced opiate receptor upregulation (Tempel et al., 1984). It is possible to use systemic morphine as an aversive unconditioned stimulus to induce conditioned taste aversion (CTA), and intraperitoneal morphine to produce conditioned place aversion (CPA) in the rat. This effect appears to be mediated by the lateral PBN (Bechara et al., 1993). However, lPBN lesions were not found to disrupt the positive reinforcing effects of morphine (as measured by conditioned place preference (CPP)), or its ability to serve as a discriminative stimulus (Nader et al., 1996).

3.4.2.2. PBN as an interoceptive and gustatory relay nucleus

PBN has been considered as a component of the respiratory, cardiovascular, and taste sensory systems (Di Lorenzo and Schwartzbaum 1982a,b; Cechetto, 1987; Chamberlin and Saper 1992; Feil and Herbert 1995; Nishijo and Norgren 1997; Karimnamazi et al. 2002), and as a component of the gustatory reward/aversion system (Shimura et al. 1997).

Most recently (Craig, 2002), lamina I afferents were recognized as serving a more general visceroceptive or interoceptive role, of which pain sensation is one manifestation. In this view, painful and normal somatosensation are carried by the same afferent fibers, but are related centrally to homeostatic requirements, such that, e.g., identical thermal stimuli are processed as normal or noxious depending on their relationship to the current homeostatic needs of the body.

The parabrachial nucleus is extensively innervated by the nucleus tractus solitarius (NTS) (Loewy & Burton, 1978). The caudal and lateral parabrachial nucleus receives ipsilateral inputs from NTS units responsive to taste and mechanical stimulation of the oral cavity (Ogawa et al., 1980; Cho et al., 2002a), and, as with other parabrachial sensory modalities, relays this gustatory input to the CeA (Block & Schwartzbaum, 1983). NTS is also considered an integrative relay for nociceptive and cardiorespiratory afferents as well as autonomic outflow (Boscan et al., 2002,

2005) related to homeostatic mechanisms such as the reflex tachycardia to acute pain. These afferents, are also relayed to the PBN (Norgren, 1978).

Norgren and Leonard (1973) found that efferents from the taste responsive region of the rat rostral NTS terminated in both medial and lateral PBN, but found taste responses only in mPBN, (i.e. ventral to the brachium), and dubbed this region the "pontine taste area." Norgren and Pfaffman (1975) later localized the pontine taste area to the caudomedial quadrant of PBN, above, below, and within the brachium (i.e. the "waist" region of PBN). The caudal and lateral parts of PBN contain units responsive to taste stimuli, and receive ipsilateral inputs from NTS units responsive to taste and mechanical stimulation of the oral cavity (Ogawa et al., 1980; Nishijo & Norgren, 1997; Cho et al., 2002). PBN fibers ascending in the central tegmental tract from the rostral lateral and ventral lateral PBN have been anatomically and antidromically confirmed to project to the thalamic taste area in the medial part of the ventrobasal complex, the ventral subthalamus, the CeA, substantia innominata, and BNST (Norgren, 1976). As in other studies, the CeA projection was found to be the most pronounced of these, completely filling that subnucleus. Feedback projections from the CeA can modulate and induce plastic changes in taste responses in both PBN, as described below.

3.4.2.3. PBN in conditioned avoidance learning

The parabrachial nucleus plays a critical role in taste- and odor-aversion learning. Taste-responsive neurons in PBN, like those in CeA and NTS (Chang & Scott, 1984), show altered responsiveness to tastants which have been associated with LiCl-induced illness (Shimura et al., 1997). CeA also plays a key role in the mediation of plasticity in the MGN (which appears to receive crossed vestibular nucleus inputs (Wepsic, 1966)) during auditory fear conditioning (Maren et al., 2001). As with MGN and other connections between CeA and lower sensory

processing areas, feedback from the CeA exerts control over plasticity of PBN gustatory responses related to the acquisition and expression of conditioned taste aversions (Smith et al., 1979; Shimura et al., 1997; Lundy & Norgren, 2001; Tokita et al., 2004), as well as taste responses in the NTS (Li et al., 2002; Cho et al., 2002b). Inactivation of PBN leads to a complete failure to learn CTA (Spector et al. 1992; Yamamoto et al. 1995; Grigson et al. 1998a,b; Reilly and Trifunovic 2000).

Systemic morphine may be used as an aversive unconditioned stimulus to induce conditioned taste aversion (CTA), and intraperitoneal morphine to produces conditioned place aversion (CPA) in rats (Bechara et al., 1993). The aversive effect appears to be mediated by lateral PBN, as it was disrupted by bilateral ibotenic acid lesions in lPBN. However, lPBN lesions were not found to disrupt the positive reinforcing effects of morphine in conditioned place preference (CPP).

3.4.2.4. PBN in arousal and attention

PBN efferents to MGN and LGN may have roles in the modulation and direction of attention and arousal. MGN relays auditory inputs to primary auditory cortex, but also demonstrates vestibular sensitivity; LGN is associated with the visual system, but also receives vestibular nuclear projections.

Unfortunately, MGN has generally been lumped together with the posterior thalamic complex in studies of PBN efferents. However, the ventromedial, magnocellular portion of MGN (MGNmc) and the suprageniculate nucleus receive vestibular nuclear projections (Kotchabhakdi, 1980), demonstrate vestibular responses (Deecke et al., 1973; Troiani et al., 1978; Blum et al., 1979), elicit sensations of self-motion upon stimulation (Hawrylyshyn et al., 1978), and project to cortical areas adjacent to primary auditory cortex (Locke, 1961; Burton & Jones, 1976;

LeDoux et al., 1991), including the inferior parietal cortex (an oft-cited candidate for primary vestibular cortex (Andersson & Gernandt, 1954; Faugier-Grimaud & Ventre, 1989)) as well as to CeA (Russchen, 1982) and LA (LeDoux & Farb, 1991). MGNmc and MGNsg are generally considered part of the auditory system, and are thought to underlie lateral amygdalar auditory responses and their plasticity (Bordi & LeDoux, 1994a,b), as MGv provides no input to LA (LeDoux et al., 1990).

A similar situation exists in LGN, which receives inputs from the vestibular nuclei (Kotchabhakdi et al., 1980; Pasquier & Villar, 1982), and contains cells responsive to vestibular stimulation (Papaioannou, 1969, 1972; Magnin & Putkonen, 1978). The vLGN is also known to receive input from the parabrachial nucleus (De Lima & Singer, 1987; Kolmac & Mitrofanis, 2000; Ozaki & Kaplan, 2006). Ozaki & Kaplan (2006) recorded from the LGN in cats and found that PBN firing rates were correlated with fluctuations in the LGN transfer ratio (the proportion of retinal spikes which are transmitted to visual cortex) across time, and that the transfer ratio could be increased by stimulation of PBN, suggesting a role for PBN in arousal- or attention-dependent the modulation of the proportion of visual inputs reaching the visual cortex.

Medial and lateral PBN also have extensive reciprocal connections with the principal LC (Sakai et al., 1977), which is considered to play important roles in arousal, attention, sleep and waking, affect, sensory processing and autonomic control (see Berridge & Waterhouse, 2003 for review). In particular, rostromedial parts of PBN have been implicated in defense reactions, as stimulation there elicits increased arterial blood pressure and vasodilation in skeletal muscles (Coote et al, 1973).

3.4.2.5. PBN as a source of autonomic and visceromotor outflow

The PBN has been identified as an important locus of autonomic control. Microstimulation of broad regions of PBN elicit strong pressor responses and bladder contractions (Wang & Ranson, 1939). Electrical stimulation of PBN or CeA can produce transient changes in blood pressure, heart rate and respiration (Coote et al., 1973; Lara et al., 1994; Harper et al., 1984), and these nuclei contain subpopulations of units which are active during all phases of respiration (Song et al., 2006). Vagal afferents from the lung arrive in NTS, and glutamate application in lPBN and KF leads to a transient hyperpnea (Chamberlin & Saper, 1994; Chamberlin, 2004), suggesting that these areas participate in the control of inspiratory tone. mPBN also receives projections from the laryngeal motocortex in the monkey (Simonyan & Jürgens, 2003), and contains units responsive during vocalization (Pieper & Jürgens, 2003). Respiration-related activity in the parabrachial nucleus is thought to be related to the close coordination of vocalization and respiration (Smotherman et al., 2006).

PBN is also reciprocally connected with the Edinger-Westphal nucleus (EW) in the rostral oculomotor complex, which is the source of preganglionic parasympathetic (pupilloconstrictor) efferents to the ciliary ganglion (Pick, 1970; Sillito and Zbrozyna, 1970; Loewy & Saper, 1978), and microstimulation of PBN has been observed to elicit pupillary dilation (Wang & Ranson, 1939).

From the above discussion and the consideration of PBN efferents (see **Table 3.1**), the picture which emerges of the organization of PB is one of loose anatomical segregation of its many afferent and efferent connections. Units participating in gustation and the coordination of respiration and vocalization appear to be most concentrated in mPBN, but show some spread into other PB subnuclei. Nociceptive functions appear most concentrated in lPBN, elPBN and KF,

and these regions seem to also be involved in the coordination of respiratory control and behavioral reactions to noxious stimuli. However, nociceptive functions of PB are in fact more diffusely represented throughout the nucleus. The majority of projections to frontal and insular cortex from PB appear to arise from mPBN and KF, although projections to septal regions appear more widespread. Finally, the vestibular responses in PB appear not to be organized in relation to any of these previously recognized systems, instead mixing almost throughout the nucleus, with a gradient from caudoventrolateral (more dense) to rostrodorsomedial (less dense) (Balaban et al., 2002).

3.5. Conclusion

Given the known relationships and bidirectional interactions between attention, affect and vestibular sensation and balance system function (reviewed briefly in **Chapter 1**), it has been proposed that vestibuloresponsive PB units serve as a relay between the vestibular nuclei and forebrain affective and associative learning mechanisms. Such a function would be consistent with the known roles of PB in nociceptive, gustatory, and visceral sensory processing. In particular, Sewards (2004) presented a cogent argument which proposed that there are separate *sensory* and *hedonic* pathways for taste which pass through separate parts of the parabrachial nucleus. These pathways engage separate groups of parabrachial efferents, one of which shows prominent primary responses to taste stimuli, and the other of which shows taste responses that are strongly affected by conditioned taste aversion. A similar hypothesis can be easily formulated for nociception, as it is known that there are two major pain-related pathways through the parabrachial nucleus: one provides a direct disynaptic connection between the spinal cord and CeA and has been interpreted as being responsible for the affective (i.e. hedonic)

aspects of pain sensation; the other provides an indirect output to thalamic nuclei long recognized as participants in the sensory aspects of pain.

It is possible that the provision of vestibular information to CeA and other higher brain regions by PB efferents acts as a substrate for the enlistment of attentional resources and autonomic mechanisms in response to challenges to spatial orientation or balance, and provides a mechanism for associative learning about particular patterns of vestibular inputs and behaviorally relevant outcomes, such as falling. It is also possible that this circuit provides a substrate for changes in balance performance under altered conditions of demand, and may be related to the effects of attention and affect on balance control and vestibular reflexes. Prior to pursuing such theories, it will first be necessary to complete the characterization of the fundamental response properties of parabrachial neurons to vestibular inputs, for example by experiments designed to clarify the exact form and origin of responses to angular rotation transients—including conclusive studies such as unit responses to whole-body motion in labyrinthectomized animals, or direct stimulation of labyrinthine afferents. Future studies of parabrachial responses to vestibular inputs in terms of such potentially interesting influences on unit responses as passive and self-generated motions, and investigations of the potential for plasticity in unit responses (as observed in PB responses to a variety of behaviorally and subjectively important stimuli) both in the context of mechanistic forms of vestibular adaptation, and more abstract forms of learning about self-motion stimuli, hold the potential to begin to unravel a number of poorly understood but clinically important relationships between alterations in balance system performance and their consequences for both motor and cognitive task performance and acute and chronic subjective consequences of real or perceived threats to spatial orientation.

The studies presented here have improved our understanding of self-motion related responses in PB by testing linear acceleration responses independently of confounding factors imposed by more complex stimulus profiles. We have shown that the majority of unit responses to linear acceleration show behavior analogous to linear acceleration responses observed in other central vestibular sites, and can be successfully accounted for by response models used to characterize responses of primary otolith afferents and second-order vestibular nuclear neurons targeted by those afferents. Other responses we observed were consistent with known responses of second and third-order central vestibular neurons hypothesized to result from convergent inputs from primary afferents or primary afferent-like second-order units with different spatial and temporal response characteristics. These studies have supported the hypothesis that self-motion related responses of PBN units are derived from the peripheral vestibular sensory organs, and have pointed to the necessity of further studies describing the basic physiological properties of parabrachial unit responses to angular velocity and acceleration.

Subnucleus	Rostral 1/3	Mid 1/3	Caudal 1/3
internal lateral		ILTh	
central lateral	VLM LH PVH ZI MnPO BNST PVTh	CeA ZI MnPO BNST	MnPO VLM
ventral lateral		LH ZI	Cx mcBN CeA ZI
dorsal lateral		MnPO PVTh BNST LH PVH DMH VLM	
external lateral	MnPO ZI	mcBN ZI	CeA MBN ZI
extreme lateral	BNST LH PVH		
medial	LH PVH ZI mcBN ILTh	LH PVH ZI VPMpc Cx VLM	Cx mcBN CeA ZI ILTh VPMpc
external medial			VPMpc Cx
caudal 'waist' area of SCP (m+vl)			Cx mcBN CeA ZI
Kolliker-Fuse	VLM	NTS VLM	SpCd

Target structures	lPBN	mPBN
Medulla		
Facial motor nucleus	++	
Nucleus ambiguus	++	
Reticular formation	++	
Midbrain		
Annular nucleus and EW	+++	+++
Ventral tegmental area	+++	
Central gray	++	++
Paranigral nucleus	++	
Substantia nigra	++	
Diencephalon		
Thalamus		
VPMpc (rostral)	++	++
VPM	++	++
Central lateral	++	
Parafascicular/centromedian	++	+
Reuniens	++	++
Central Inferior		+
Hypothalamus		
Dorsomedial	+++	+
Lateral	++	++
Ventromedial	++	
Supramammillary	++	+
Infundibular	++	
Dorsal	+	+
Supraoptic	+	
Paraventricular	+	
Telencephalon		
CeA	++++	++
BNST (lat)	++++	++
Triangular septal nucleus	++	+
Medial preoptic	+	
Lateral preoptic	+	
Nuc. of anterior commisure	+	+
Nucleus accumbens	+	

Table 3.1 Efferent connections of PB in the rat and monkey

Left: Rostrocaudal topography of the efferents of PB subdivisions in the rat. (Fulwiler & Saper, 1984). *Right*: efferent projections of lPBN and mPBN in the cynomolgus monkey as described by Pritchard et al. (2000). These tables show that there is considerable overlap in the projection fields of various subnuclei in both species.

APPENDIX A

Abbreviations Used in the Text

BNST	Bed nucleus of the stria terminalis
CeA	Central nucleus of the Amygdala
COM	Center of mass
Cx	Cortex
DMH	Dorsomedial hypothalamic nucleus
EW	Edinger-Westphal nucleus
ILTh	Intralaminar thalamic nuclei
LH	Lateral hypothalamus
mcBN	Magnocellular thalamic basal nucleus
MGmc	Magnocellular medial geniculate nucleus
MGsg	Supragenicuiate (medial geniculate) nucleus
MnPO	Median preoptic nucleus
PVH	Paraventricular hypothalamic nucleus
PVTh	Paraventricular thalamic nucleus
SMD	Space and motion discomfort
SpCd	Spinal cord
VLM	Ventrolateral medulla
VPMpc	Ventroposteromedial nucleus of the thalamus, parvocellular part
ZI	Zona incerta

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